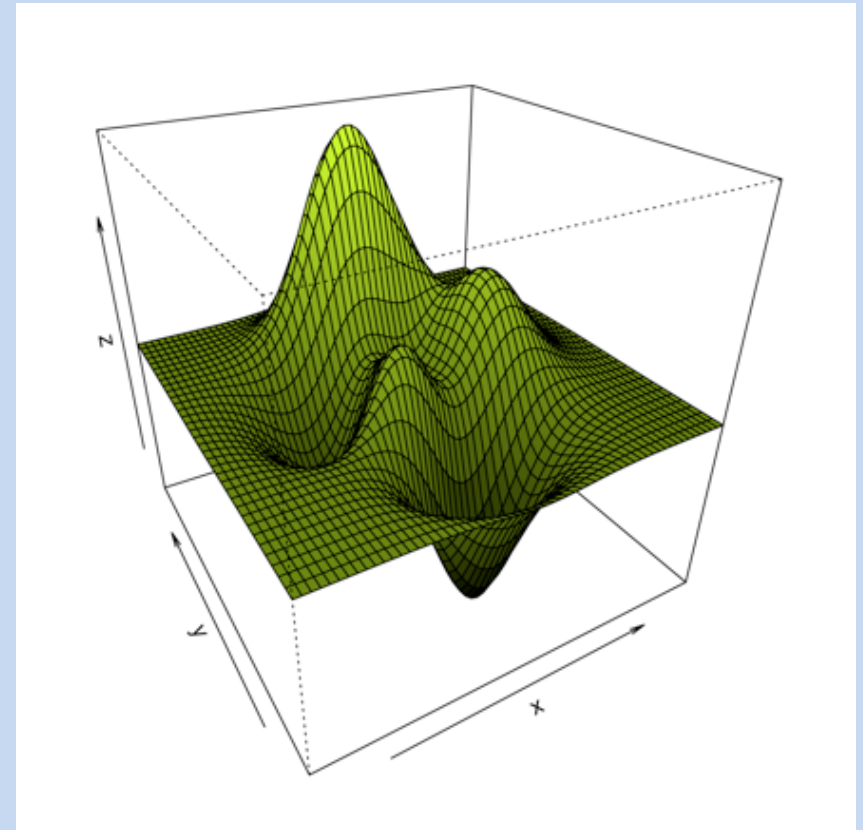
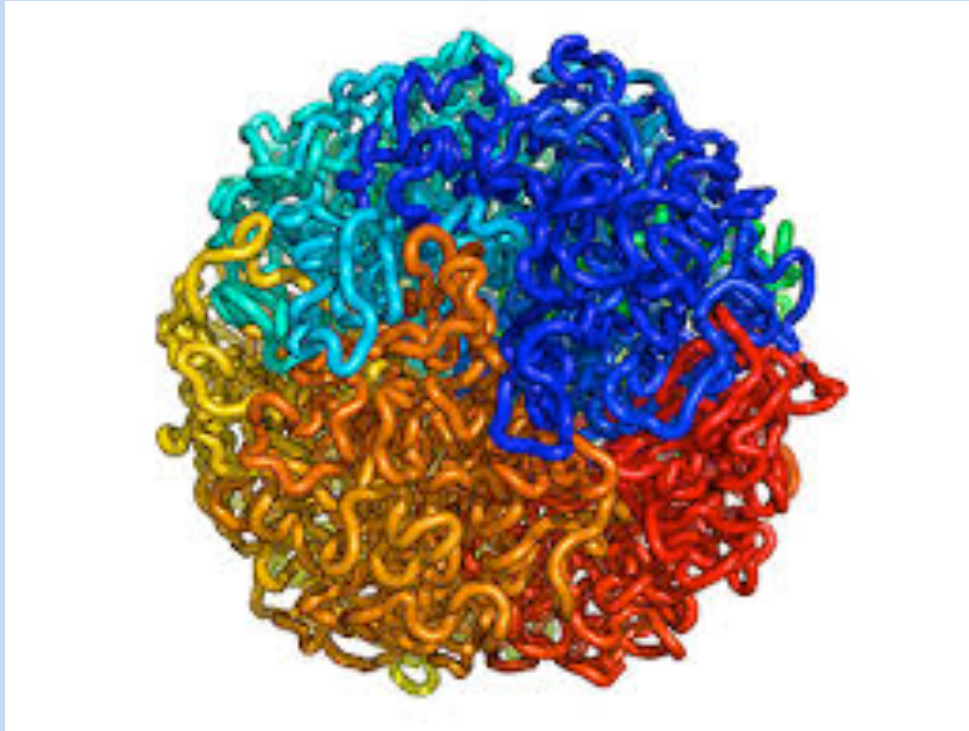


# **N-terminal acetyltransferases and Translation**

Gholson J. Lyon, M.D. Ph.D.





“There are ~12 billion nucleotides in every cell of the human body, and there are ~25-100 trillion cells in each human body. Given somatic mosaicism, epigenetic changes and environmental differences, no two human beings are the same, particularly as there are only ~7 billion people on the planet”.

# Chromosomes Are Functionally Structured

Telomere

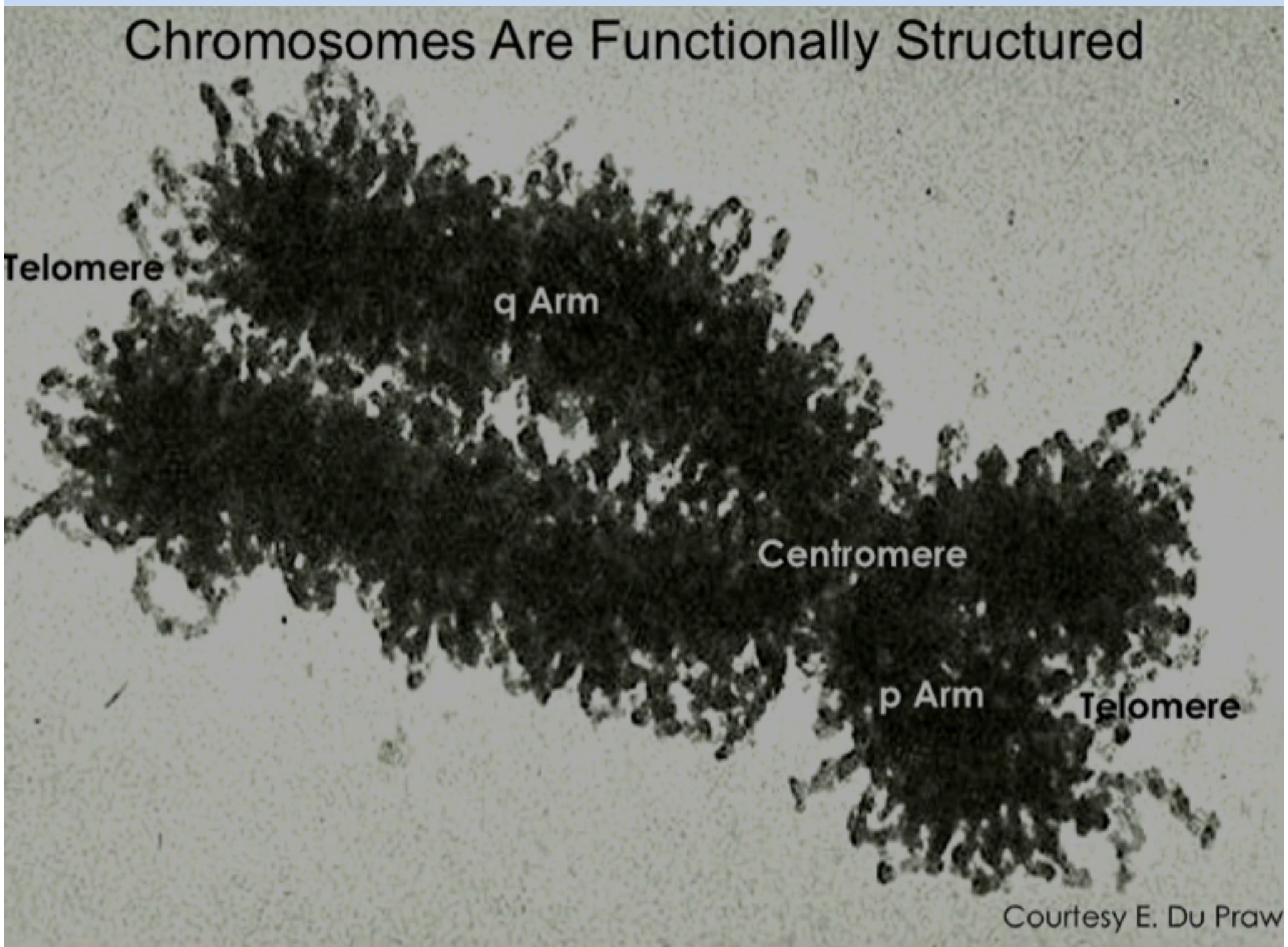
q Arm

Centromere

p Arm

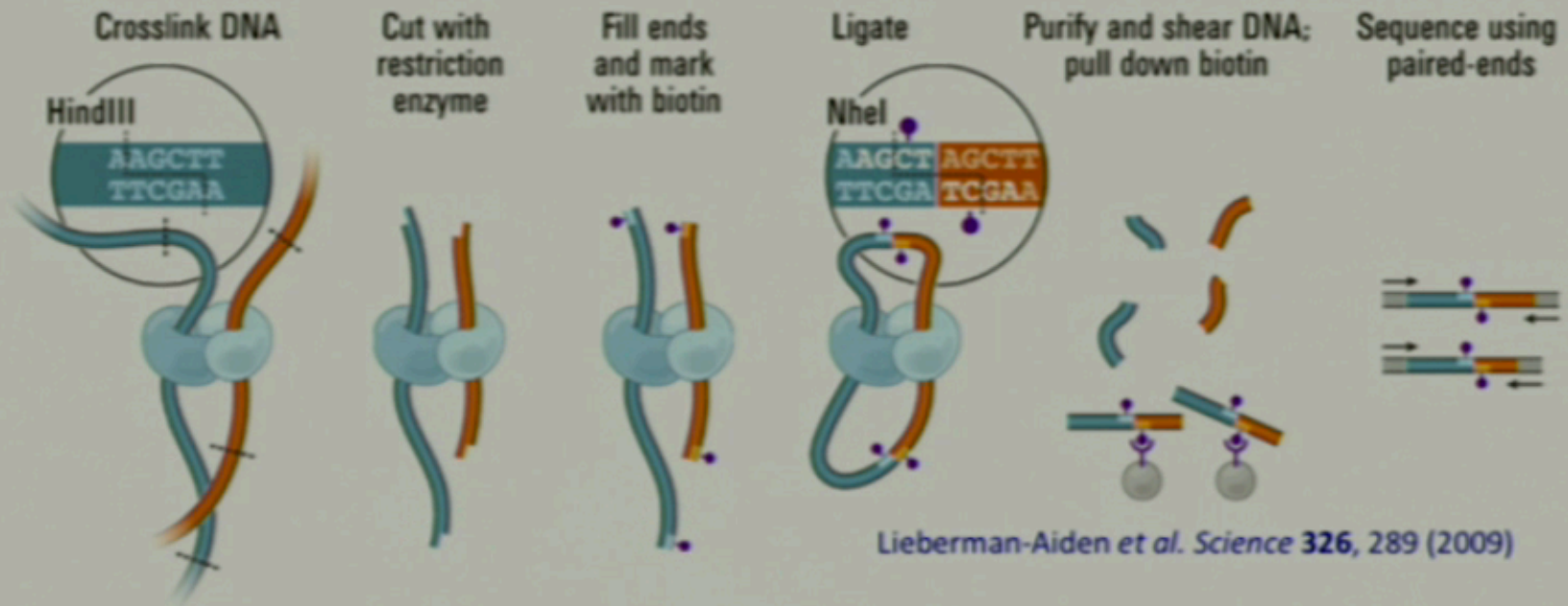
Telomere

Courtesy E. Du Praw

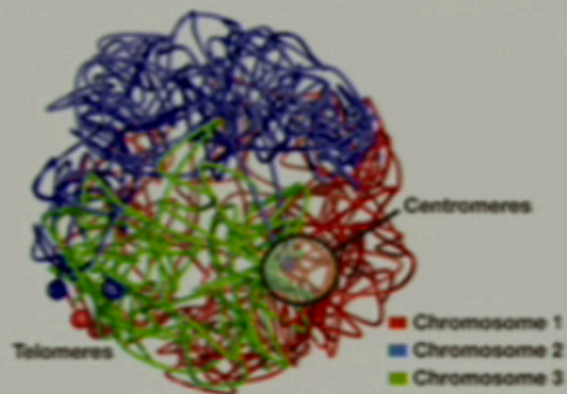




## Hi-C schematic

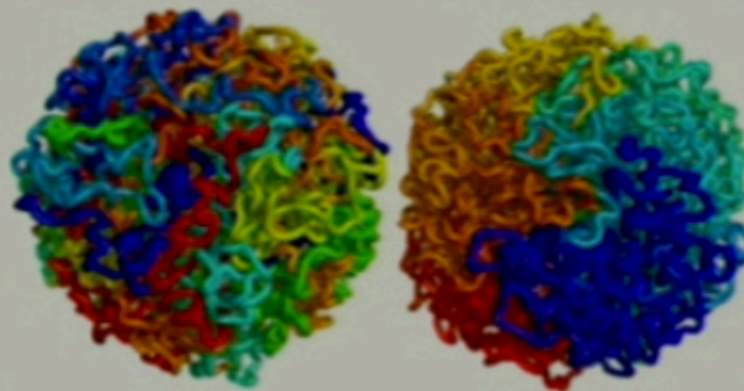


## 3D model of yeast genome



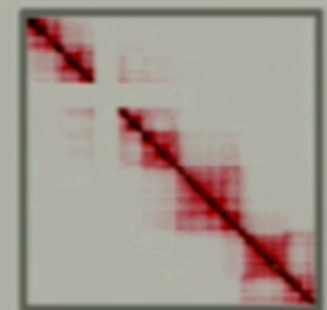
Ken-ichi Noma

## The Fractal Globule model



Job Dekker

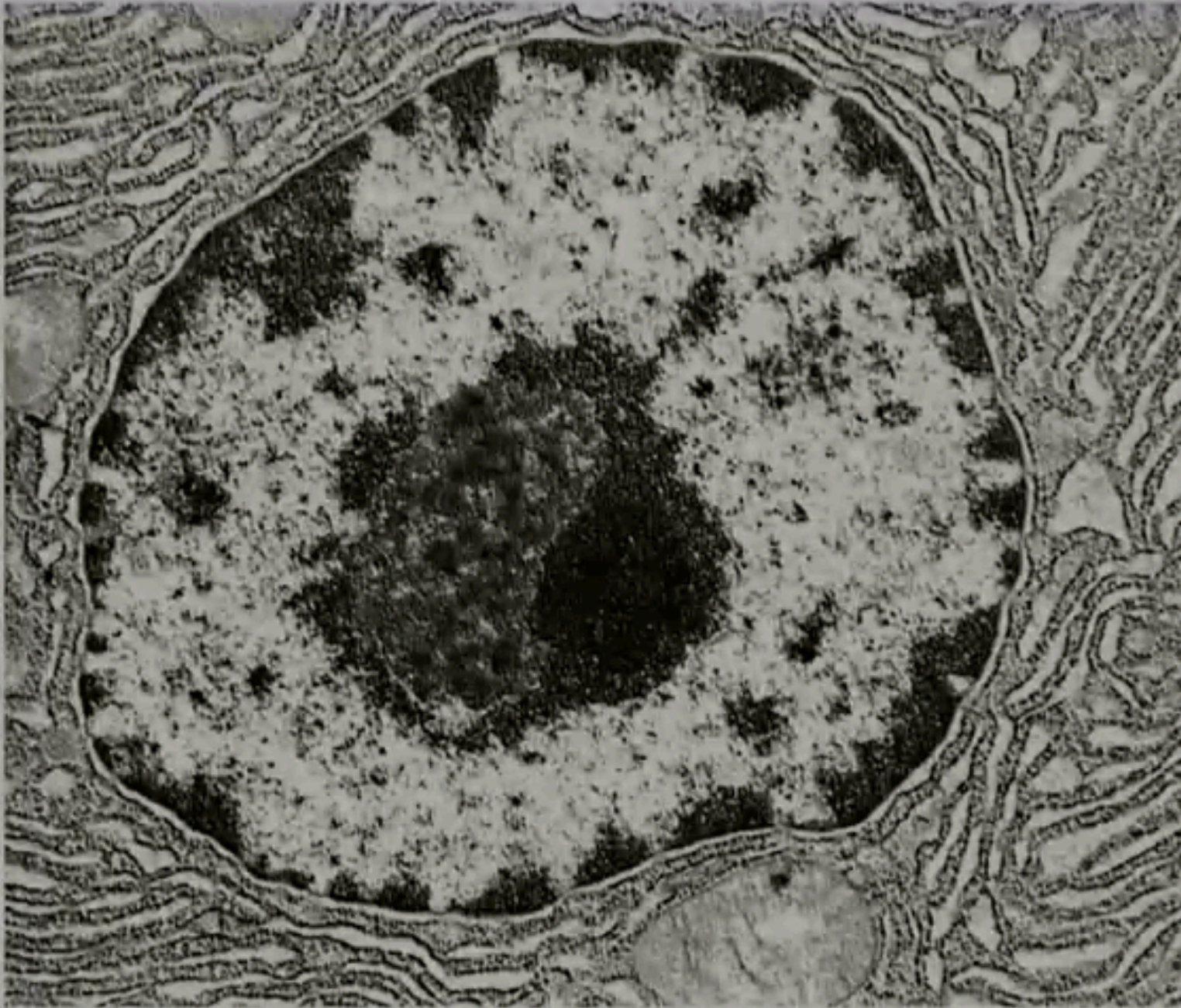
Hi-C evidence of Mb scale compacted structural domains



Nora *et al. Nature* (2012)



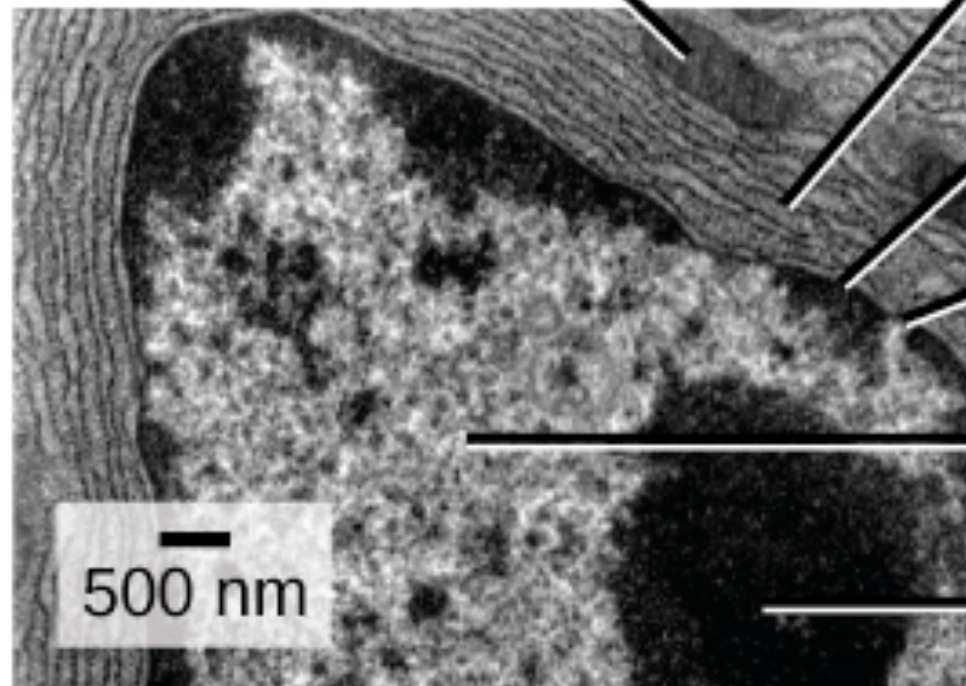
# The Nucleus is Functionally Compartmentalized



Courtesy Kenneth M. Bart

Mitochondrion overlaying  
part of the RER

Rough endoplasmic reticulum



Nuclear envelope

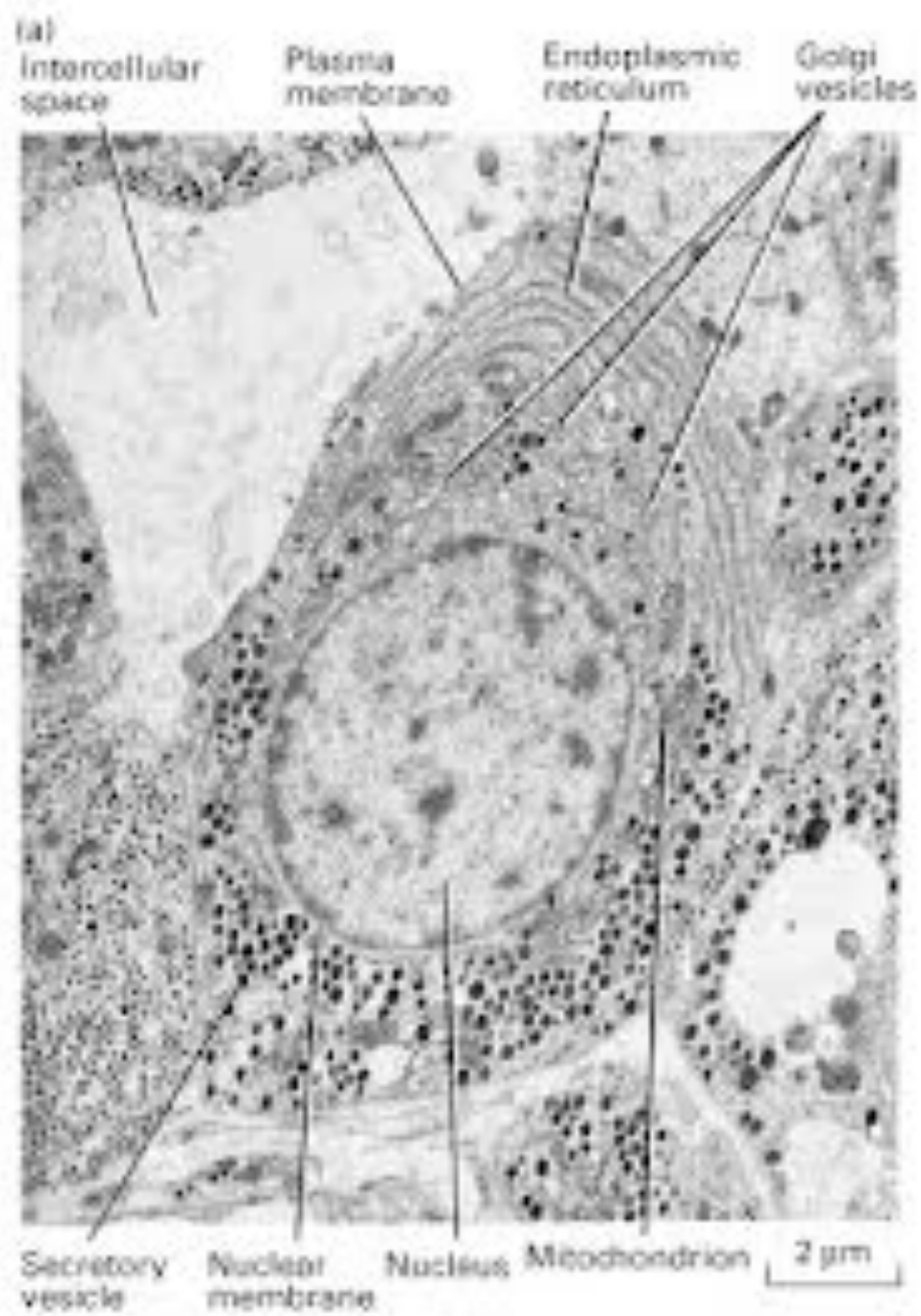
Nuclear pore

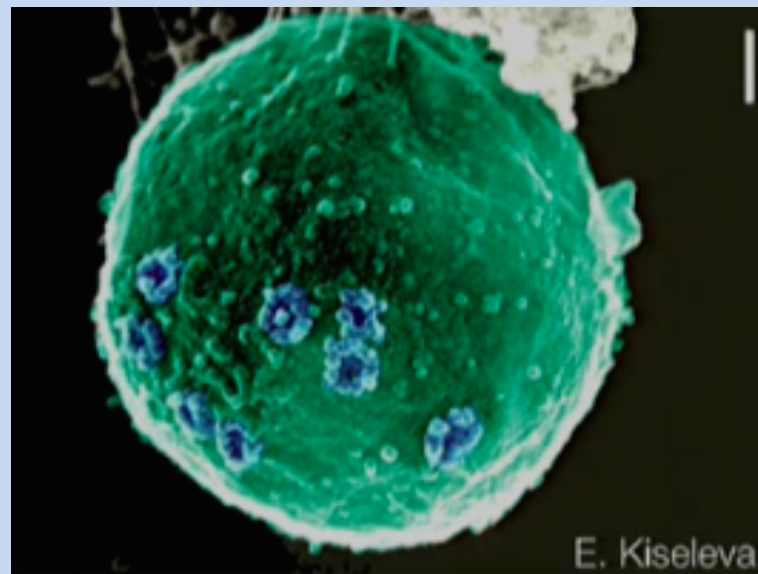
Nucleus

Nucleolus

500 nm



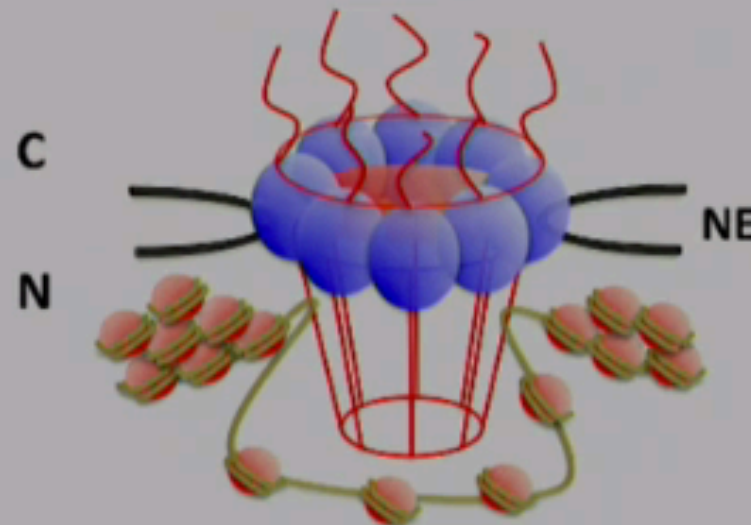
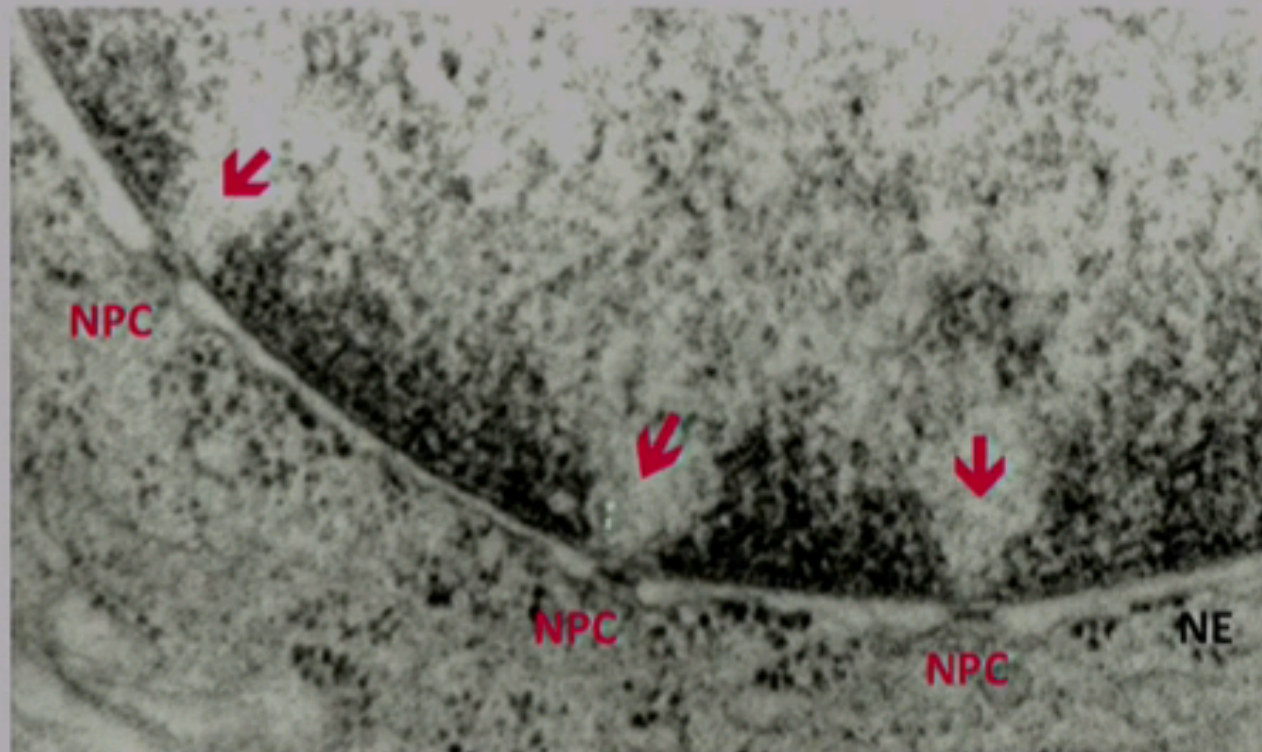


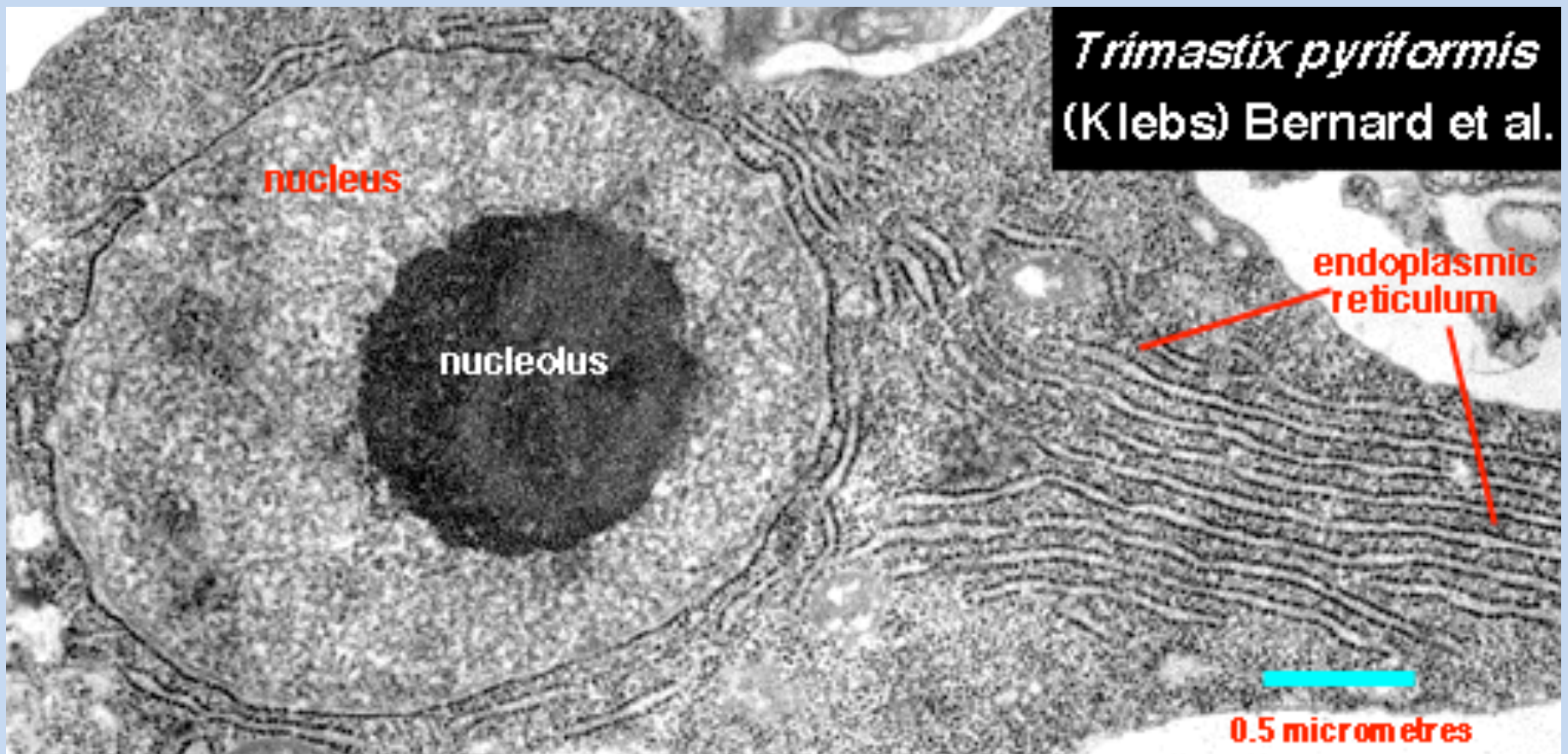


Nuclear pores in a yeast nucleus



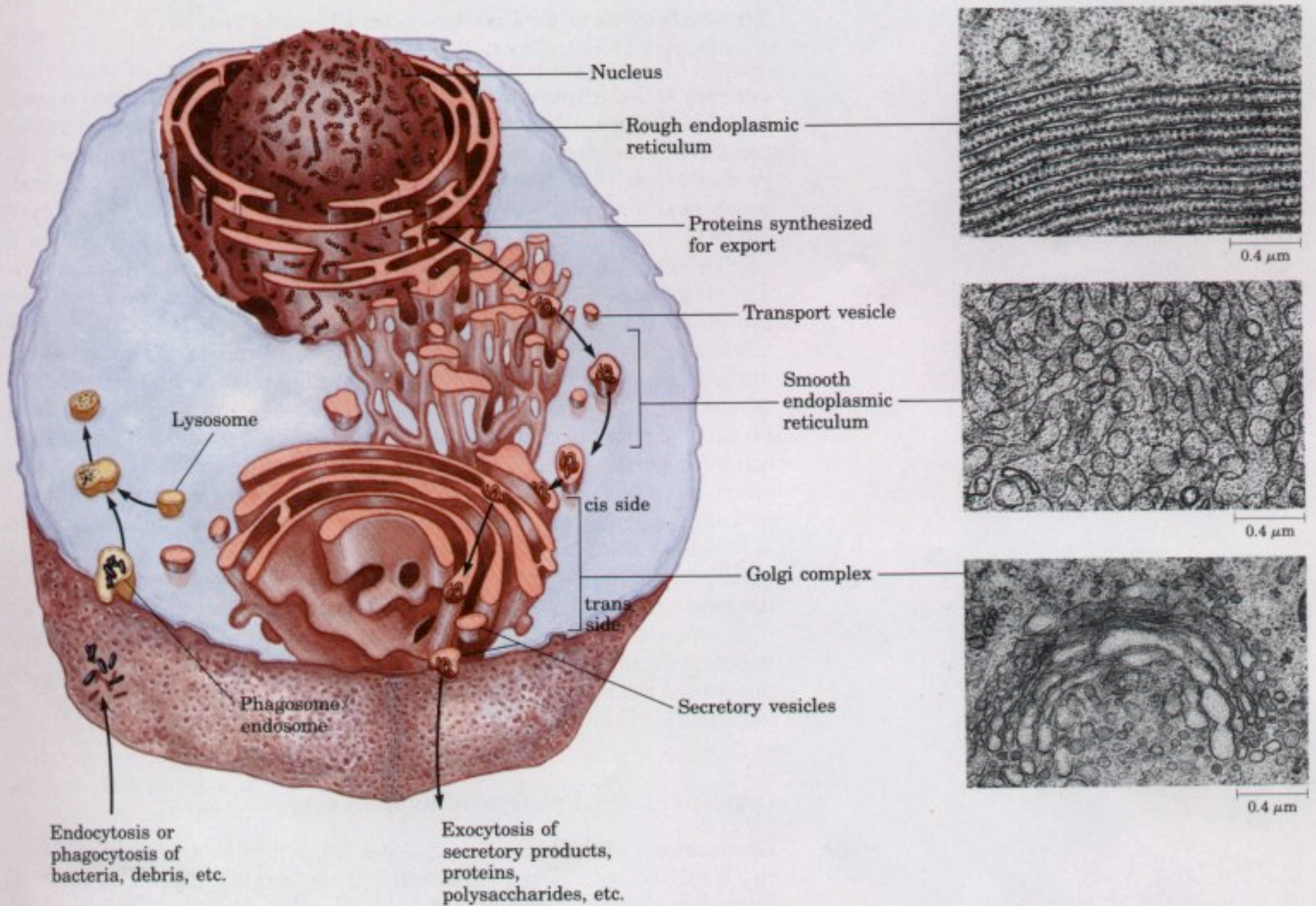
Nuclear Pore proteins appear to function as scaffolds for open/active chromatin domains



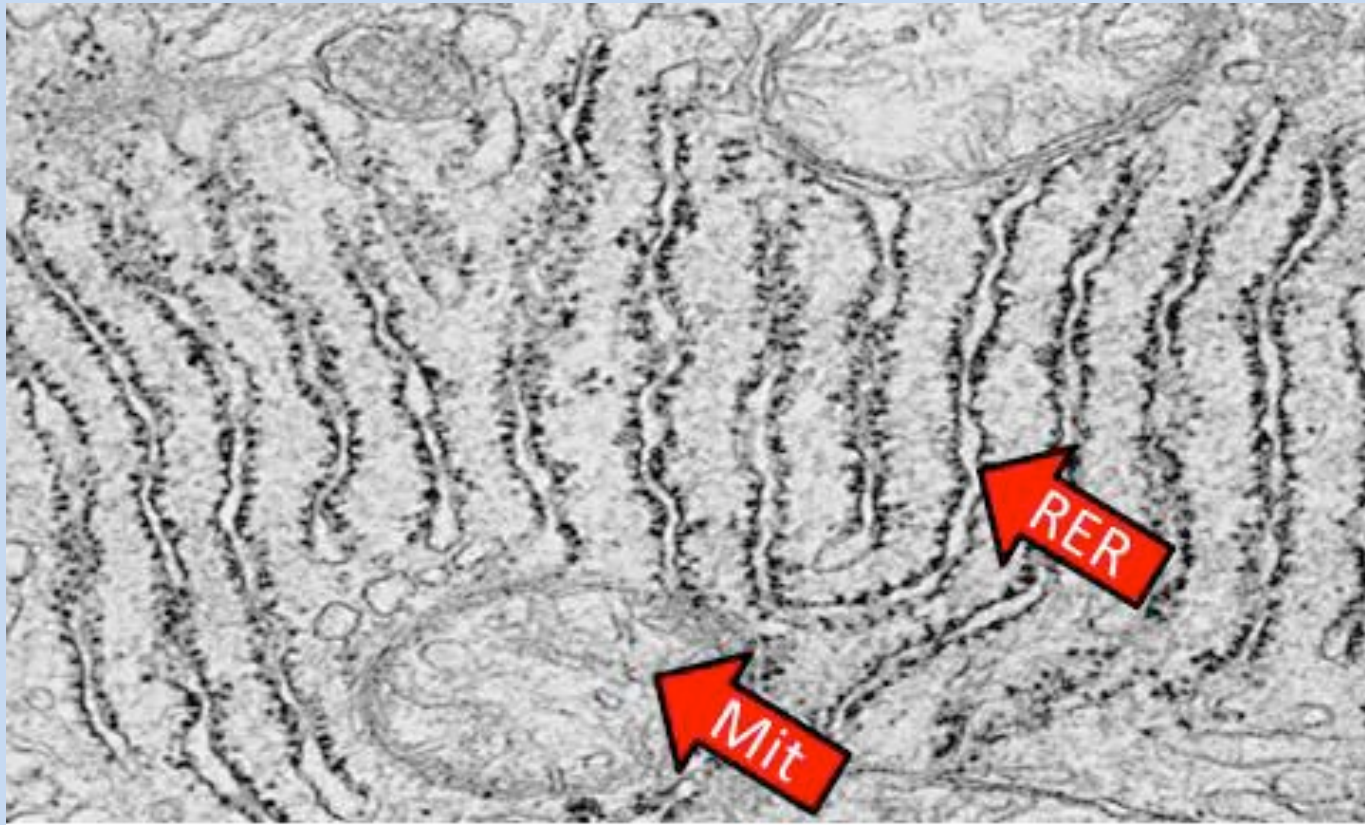


Trimastix pyriformis is a quadriflagellate, free-living, bacterivorous heterotrophic nanoflagellate from anoxic freshwaters that lacks mitochondria

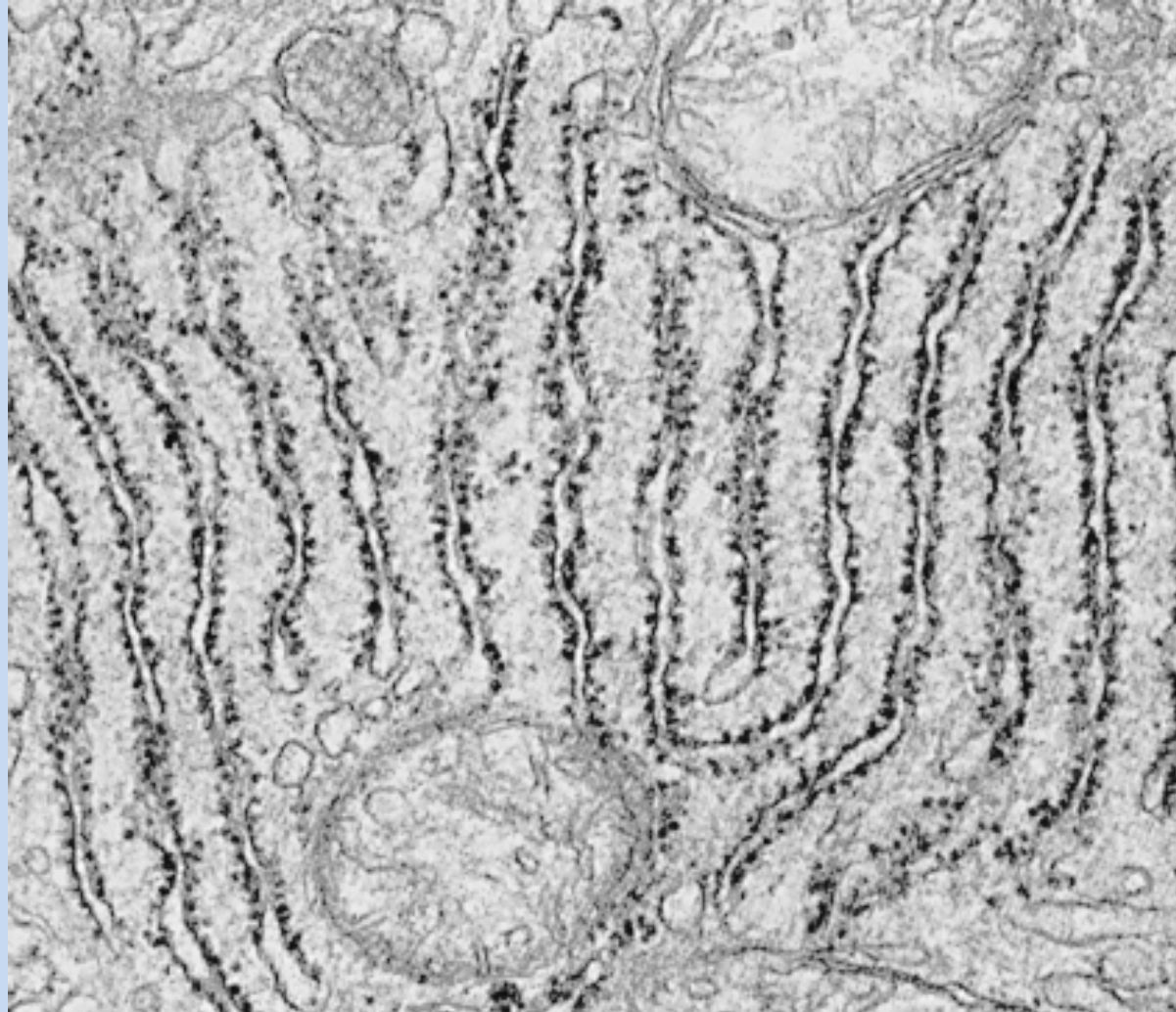


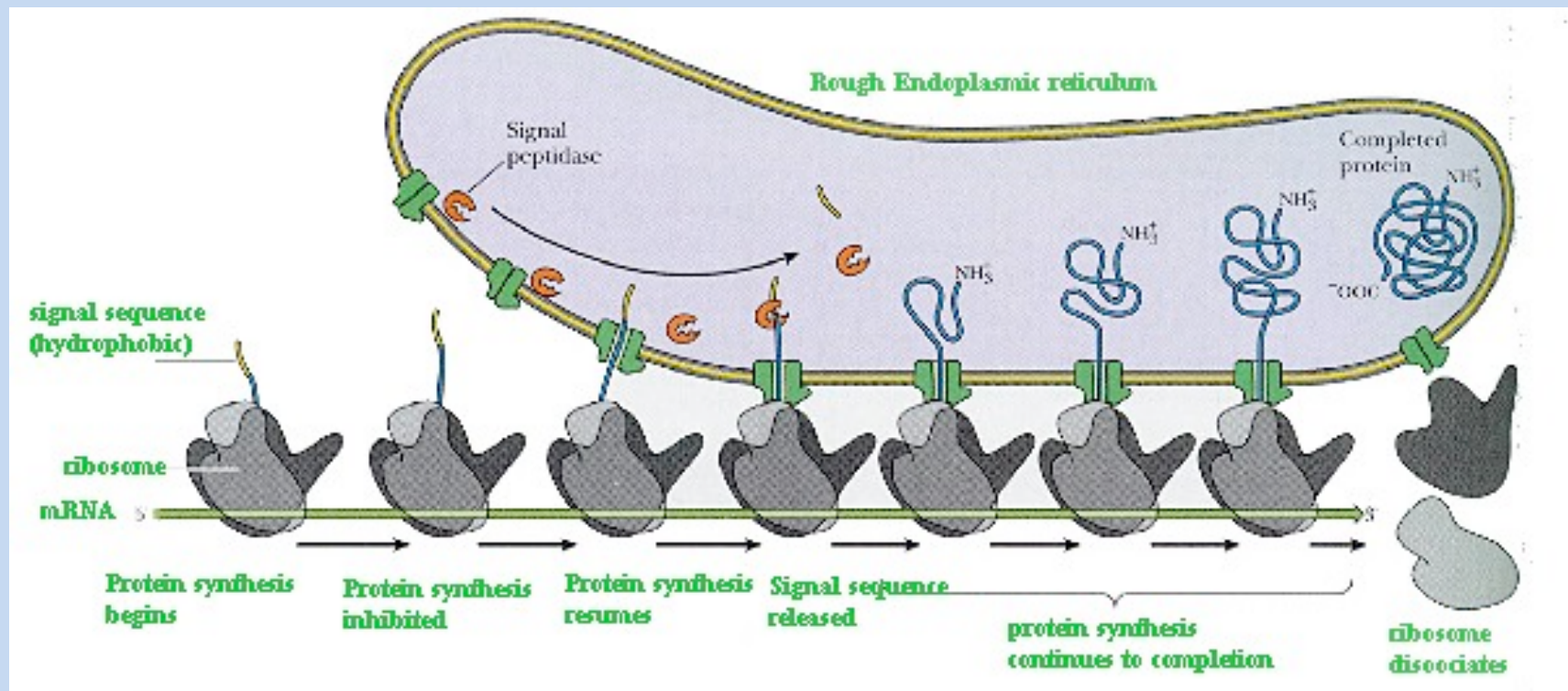




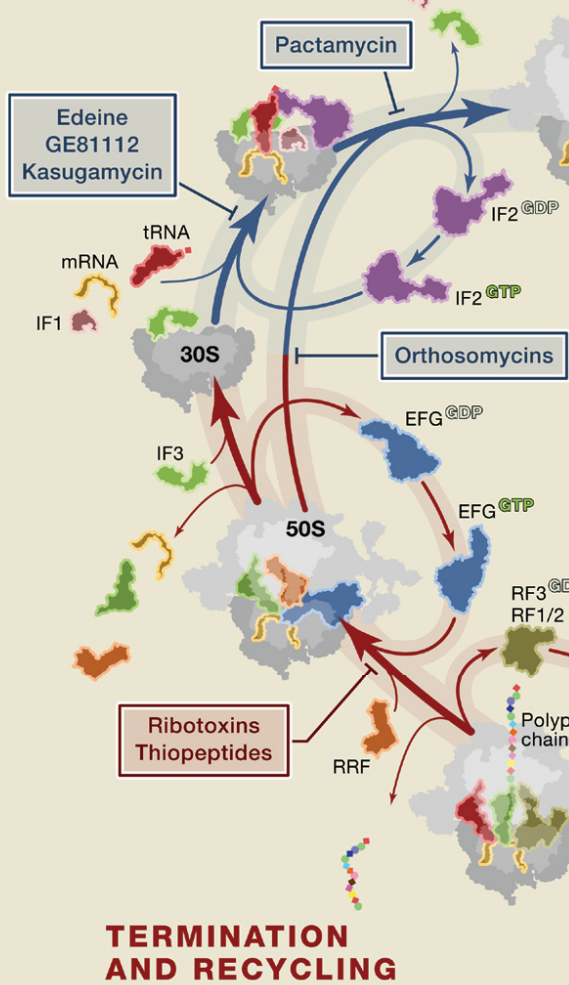




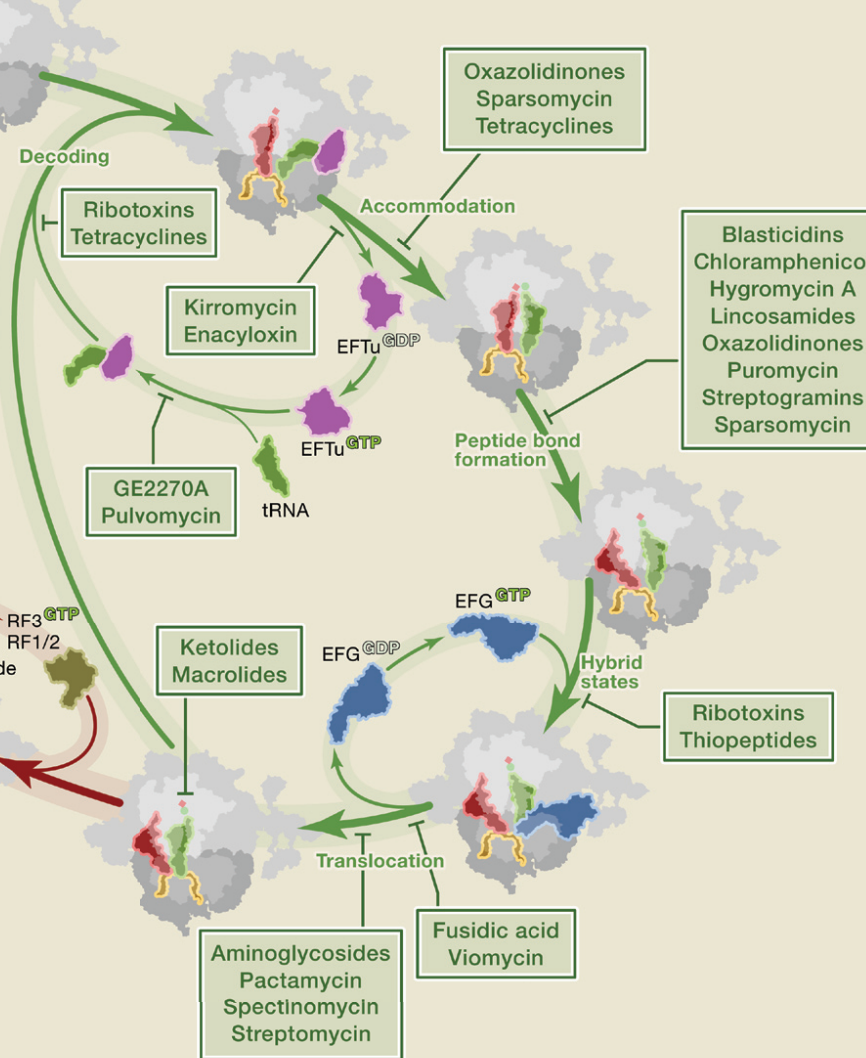




## INITIATION

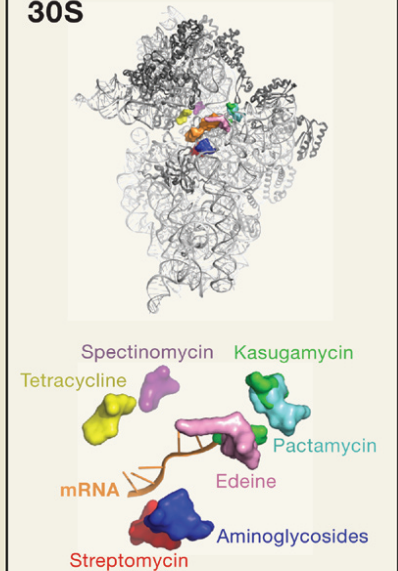


## ELONGATION

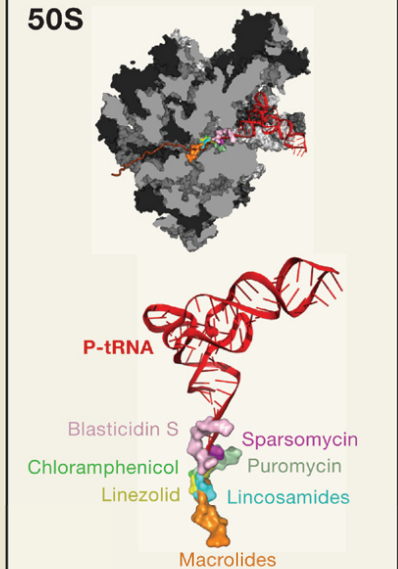


## TERMINATION AND RECYCLING

### 30S



### 50S





# **SnapShot: Antibiotic Inhibition of Protein Synthesis II**

# Cell

Daniel Sohmen,<sup>1</sup> Joerg M. Harms,<sup>2</sup> Frank Schlünzen,<sup>3</sup> and Daniel N. Wilson<sup>1,4</sup>

<sup>1</sup>University of Munich, Germany; <sup>2</sup>MPSD, University of Hamburg, Germany; <sup>3</sup>DESY, Hamburg, Germany; <sup>4</sup>CiPS-M, Munich, Germany

The translational apparatus is one of the major targets for antibiotics in the bacterial cell. Antibiotics predominantly interact with the functional centers of the ribosome, namely the messenger RNA (mRNA)-transfer RNA (tRNA) decoding region on the 30S subunit, the peptidyltransferase center on the 50S subunit, or the ribosomal exit tunnel through which the nascent polypeptide chain passes during translation. Protein synthesis can be divided into three phases: initiation, elongation, and termination/recycling.

## 1. Initiation

Translation initiation operates through a 30S initiation complex (30S-IC), consisting of the 30S, mRNA, initiator fMet-tRNA, and three initiation factors, IF1, IF2, and IF3. Subsequently, the 30S-IC associates with the 50S, which releases the initiation factors (IFs) and leaves the initiator-tRNA at the peptidyl-tRNA-binding site (P site), base-paired to the start codon of the mRNA. The antibiotics edeine, GE81112, and kasugamycin interfere with 30S-IC assembly, whereas formation of a functional 70S-IC is blocked by the orthosomycins and pactamycins.

## 2. Elongation

The elongation phase involves the movement of tRNAs in a cyclic fashion through the three tRNA-binding sites (A→P→E) on the ribosome. The first step in the cycle involves the delivery of the aminoacyl-tRNA (aa-tRNA) to the aa-tRNA-binding site (A site), which is facilitated by the elongation factor EF-Tu•GTP. Hydrolysis of GTP by EF-Tu leads to its dissociation from the ribosome, allowing aa-tRNA accommodation. Peptide-bond formation then proceeds, transferring the entire polypeptide chain from the P-tRNA to the aa-tRNA in the A site. The ribosome now has a peptidyl-tRNA at the A site and an uncharged tRNA at the P site. This ribosomal state is highly dynamic with the tRNAs oscillating between classical (A and P sites) and hybrid states (A/P and P/E sites on 30S/50S). EF-G binds to the ribosome, which stabilizes the tRNAs in hybrid states, hydrolyzes GTP to GDP, and catalyzes the translocation reaction. Translocation shifts the peptidyl-tRNA from the A/P hybrid state to the P site and the deacylated tRNA from the P/E to the exit site (E site). EF-G•GDP dissociates leaving the A site vacant for the next incoming aa-tRNA. The majority of antibiotics targeting translation inhibit a step associated with the elongation phase. Most antibiotics binding to the 30S perturb either tRNA binding, decoding, or translocation, whereas antibiotics targeting the 50S inhibit peptide-bond formation, extension of the polypeptide chain, or stable binding of translation factors. In contrast, some antibiotics interact directly with elongation factors and prevent them from dissociating from the ribosome.

## 3. Termination/Recycling

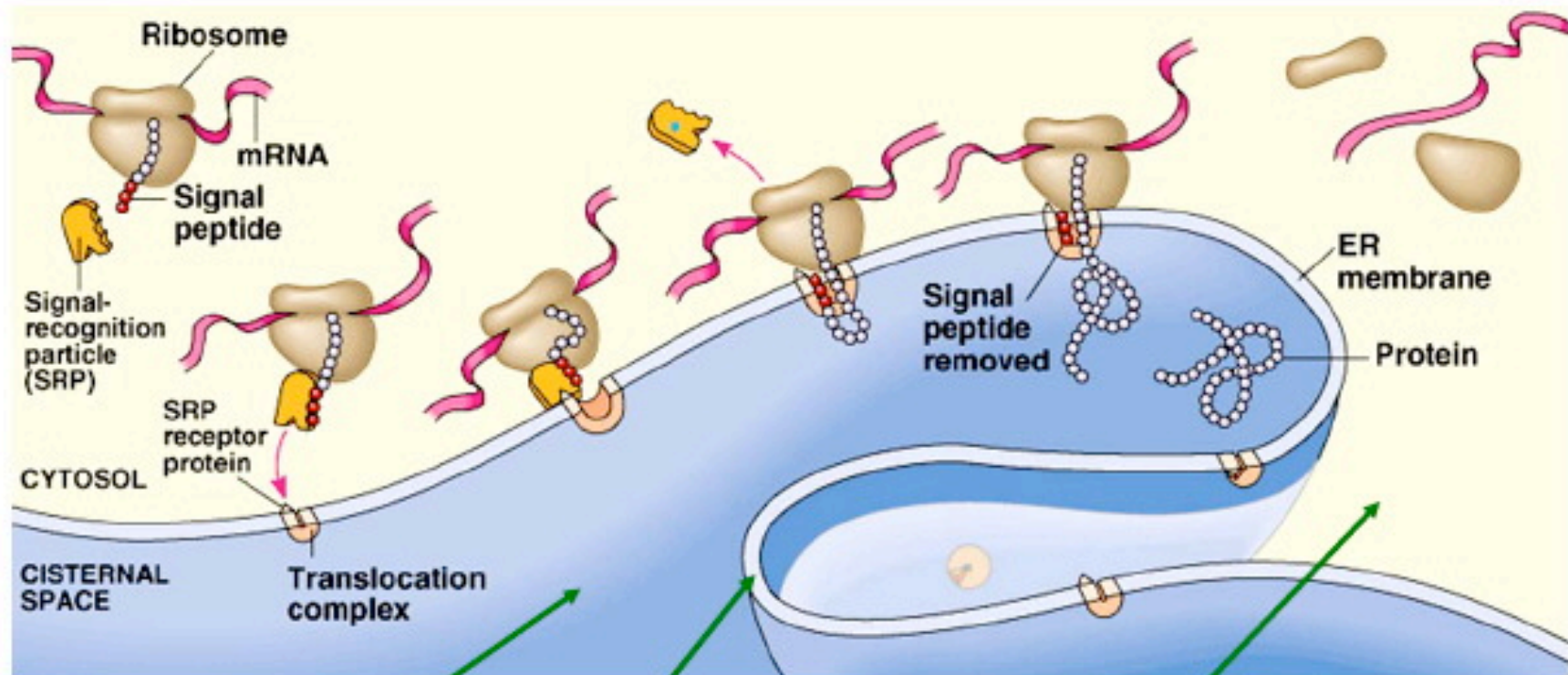
Arrival of an mRNA stop codon in the A site of the ribosome signals the termination of protein synthesis. Release factor 1 (RF1) or RF2 binds to the ribosome and hydrolyzes the peptidyl-tRNA bond, releasing the translated polypeptide chain from the ribosome. RF1/2 is recycled from the ribosome by RF3 in a GTP-dependent fashion. The ribosome is then split into subunits by the concerted action of EF-G and ribosome recycling factor (RRF), thus recycling the components for the next round of translation. There are no antibiotics that specifically inhibit termination and ribosome recycling; however antibiotics that target EF-G, such as  $\alpha$ -sarcin, fusidic acid, and thiopeptides, also inhibit ribosome recycling.

## Abbreviations

30S, small ribosomal subunit; 50S, large ribosomal subunit; aa-tRNA, aminoacyl-tRNA; A site, aa-tRNA-binding site; E site, exit site for tRNA; EF, elongation factor; IF, initiation factor; mRNA, messenger RNA; P-tRNA, peptidyl-tRNA; P site, P-tRNA-binding site; PTC, peptidyltransferase center; RRF, ribosome recycling factor; RF, release factor; tRNA, transfer RNA.

	Class	Name	Target	Mechanism
Small (30S) Ribosomal Subunit	Aminoglycosides	Apramycin, gentamycin, hygromycin B, kanamycin, neomycin, paromomycin, tobramycin	Elongation (translocation)	Most aminoglycoside antibiotics induce translational misreading by promoting binding of near-cognate tRNAs, but the biological effect is probably due to inhibition of the translocation reaction and promotion of back-translocation.
	Edeine	Edeine A	Initiation (fMet-tRNA binding)	Edeine prevents binding of the initiator tRNA to the 30S subunit in an mRNA-dependent manner.
	GE81112	GE81112	Initiation (fMet-tRNA binding)	GE81112 inhibits binding of the initiator tRNA to the 30S subunit in an mRNA-independent manner.
	Kasugamycin	Kasugamycin	Initiation (fMet-tRNA binding)	Kasugamycin inhibits translation initiation of canonical mRNAs by binding in the path of the mRNA and preventing stable interaction of the initiator tRNA with the start codon.
	Pactamycin	Pactamycin	Initiation, elongation (translocation)	Early data suggest that pactamycin allows 30S but not 70S initiation complex formation, whereas recently pactamycin was shown to inhibit translocation in a tRNA-mRNA-dependent manner.
	Spectinomycin	Spectinomycin	Elongation (translocation)	Spectinomycin binds to the neck of the small subunit and prevents the relative movement of the head and body that is needed for the completion of translocation.
	Streptomycin	Streptomycin	Elongation (misreading)	Streptomycin increases affinity of tRNA for the A site, has a modest inhibitory effect on translocation, promotes back-translocation, and induces high-level translational misreading.
	Tetracyclines/glycylcyclines	Doxycycline, minocycline, tetracycline/tigecycline	Elongation (tRNA delivery)	Tetracyclines prevent stable binding of the EF-Tu-tRNA-GTP ternary complex to the ribosome and inhibit accommodation of A-tRNAs upon EF-Tu-dependent GTP hydrolysis.
	Viomycin	Capreomycin, viomycin	Elongation (translocation)	Viomycin locks tRNAs in a hybrid-site translocation intermediate state, preventing conversion by EF-G into a posttranslocation state.
Large (50S) Ribosomal Subunit	Blasticidins	Blasticidin S	Elongation (peptidyltransferase)	Two molecules of Blasticidin S mimic the C74 and C75 of P-tRNA and in doing so inhibit peptide bond formation by preventing tRNA binding at the P site of the peptidyltransferase center (PTC).
	Chloramphenicols	Chloramphenicol	Elongation (peptidyltransferase)	Chloramphenicol binds at the A site of the PTC where it perturbs placement of A site tRNA and thus prevents peptide bond formation.
	Hygromycin A	Hygromycin A	Elongation (peptidyltransferase)	Hygromycin A overlaps in its binding site with chloramphenicol and inhibits peptide bond formation by inhibiting the placement of the A site tRNA at the PTC.
	Ketolides	Cethromycin (ABT-773), telithromycin	Elongation (nascent chain egress)	Ketolides are derivatives of macrolides where the ketone group replaces the C3 sugar. Ketolides exhibit the same mechanism of action as macrolides by blocking the ribosomal tunnel and inducing peptidyl-tRNA drop-off.
	Lincosamides	Clindamycin, lincomycin	Elongation (peptidyltransferase)	Lincosamides span across both A and P sites of the PTC and inhibit binding of tRNA substrates at both these sites.
	Macrolides	Azithromycin, carbomycin A, clarithromycin, erythromycin, spiramycin, troleandomycin, tylosin	Elongation (nascent chain egress)	Macrolides bind within the ribosomal tunnel and prevent elongation of the nascent polypeptide chain, which leads to peptidyl-tRNA drop-off. Some macrolides, such as carbomycin A and tylosin, also directly inhibit peptide bond formation.
	Orthosomycins	Avilamycin, evernimicin	Initiation (70S-IC formation)	Orthosomycins interact with the large subunit and prevent the joining of the 30S-IC with the large subunit to form the 70S-IC in an IF2-dependent manner.
	Oxazolidinones	Eperezolid, linezolid	Elongation (peptidyltransferase)	Linezolid binds at the PTC in a position overlapping the aminoacyl moiety of A-tRNA, preventing tRNA accommodation and peptide bond formation.
	Puromycin	Puromycin	Elongation (peptidyltransferase)	Puromycin is a structural analog of the 3' end of a tRNA, which binds at the A site of the PTC and triggers premature release of the nascent polypeptide chain.
	Ribotoxins	$\alpha$ -sarcin	Elongation (translocation), recycling	$\alpha$ -sarcin cleaves the <i>E. coli</i> 23S rRNA on the 3' side of G2661, abolishing the ribosome's ability to stimulate the GTP hydrolysis activity of translation factors, such as EF-G.
	Sparsomycin	Sparsomycin	Elongation (peptidyltransferase)	Sparsomycin binds at the PTC where it blocks tRNA binding at the A site, while promoting translocation and stabilization of tRNA at the P site.
	Streptogramins A/B	Dalfopristin/quinuapristin, virginiamycin M/S	Elongation (peptidyltransferase)	Streptogramins A and B act synergistically to inhibit translation. S <sub>1</sub> compounds bind at the PTC overlapping A and P sites, whereas S <sub>2</sub> compounds bind within the tunnel in a similar location to macrolides.
	Thiopeptides	Micrococцин, nocathiacin, nosiheptide, thiazomycin, thiostrepton	Elongation (translocation), Recycling	Thiopeptide antibiotics inhibit translocation by blocking the stable binding of EF-G to the ribosome. Thiopeptides also inhibit the action of translational GTPases, such as IF2 and EF-Tu.
	Fusidic acid	Fusidic acid	Elongation (translocation)	Fusidic acid stabilizes EF-G on the ribosome by allowing binding and GTP hydrolysis, but not Pi release, nor the associated conformational changes in EF-G necessary for dissociation.
Elongation Factors	GE2270A-like thiopeptides	Amythiamicins, GE2270A, thiomuracins	Elongation (tRNA delivery)	The GE2270A-like thiopeptides, like pulvomycin, prevent ternary complex formation by binding to EF-Tu and blocking interaction of EF-Tu with aminoacyl-tRNAs. GE2270A and pulvomycin have distinct but overlapping binding sites on EF-Tu.
	Kirromycins and enacyloxins	Aurodox, kirromycin, and enacyloxin IIa	Elongation (tRNA delivery)	These drugs trap EF-Tu on the ribosome by allowing GTP hydrolysis but preventing Pi release and the conformational changes in EF-Tu that are necessary for tRNA release and EF-Tu dissociation.
	Pulvomycin	Pulvomycin	Elongation (tRNA delivery)	Pulvomycin prevents ternary complex formation by binding to EF-Tu and blocking interaction of EF-Tu with aminoacyl-tRNAs.

## Insertion of Polypeptide Chains into the Endoplasmic Reticulum



lumen

membrane of  
rough e.r.

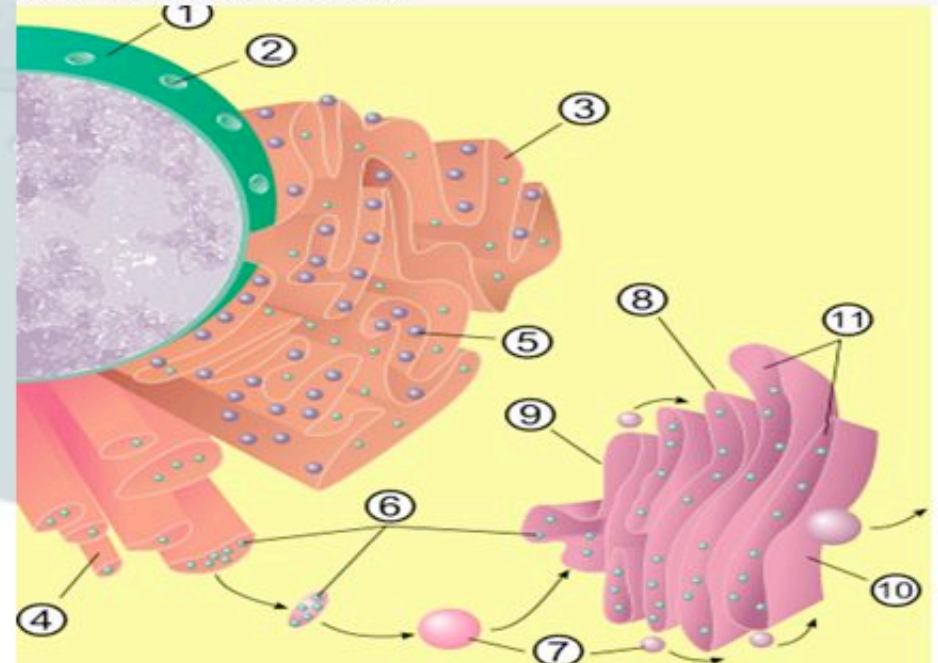
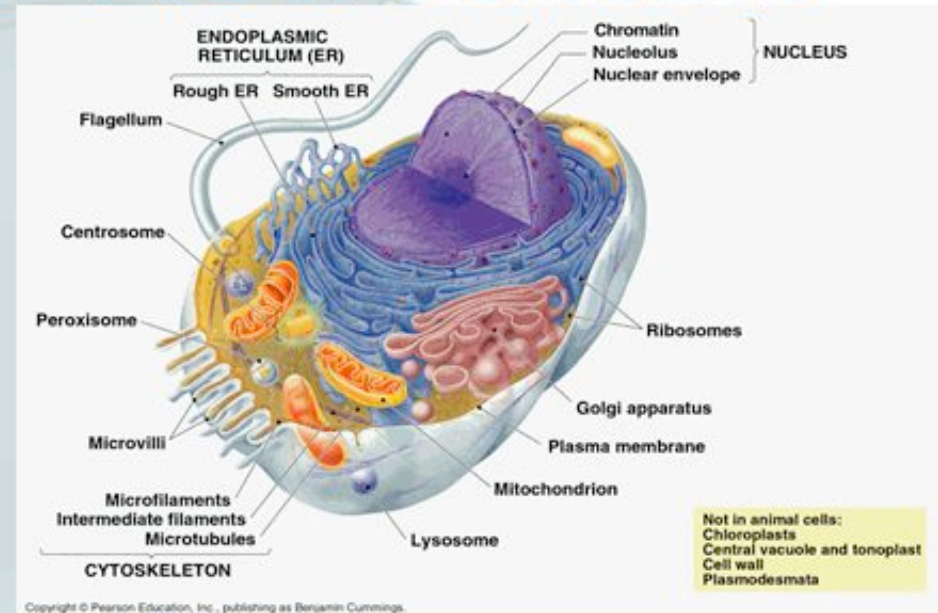
cytoplasmic  
matrix





# Endoplasmic Reticulum

- interconnected flattened tubular tunnels continuous with outer membrane of nucleus
- all eukaryotic cells contain ER
- responsible for communication in cell
- part of **endomembrane system**
- all membranous organelles belong to system
- **Rough**
  - attached ribosomes
  - makes appear spotty
  - takes in proteins made on ribosomes so cannot escape into cytoplasm
- **Smooth**
  - no attached ribosomes → smooth
  - not involved in protein synthesis
  - **steroid production**
- contains enzymes required to detoxify wide variety of organic molecules
- storage site for calcium



# Structure of the Mammalian Ribosome-Sec61 Complex to 3.4 Å Resolution

Rebecca M. Voorhees,<sup>1,2,\*</sup> Israel S. Fernández,<sup>1,2</sup> Sjors H.W. Scheres,<sup>1</sup> and Ramanujan S. Hegde<sup>1,\*</sup>

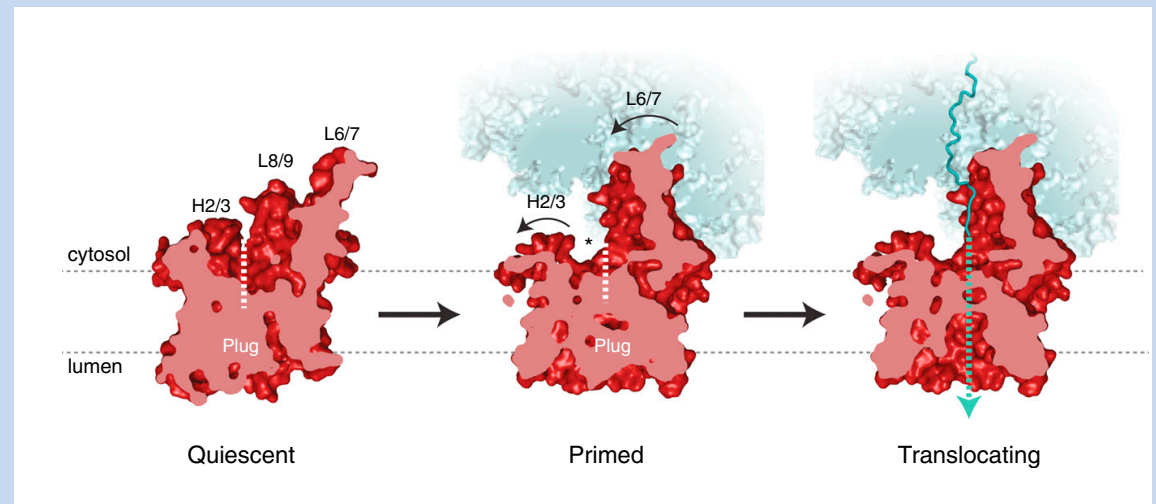
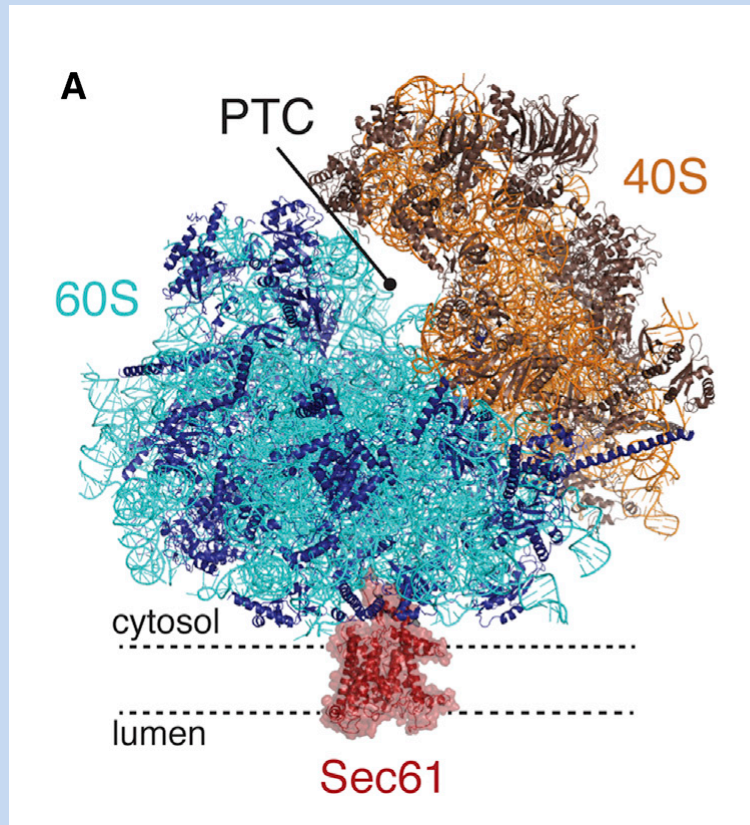
<sup>1</sup>MRC Laboratory of Molecular Biology, Francis Crick Avenue, Cambridge, CB2 0QH, UK

<sup>2</sup>Co-first authors

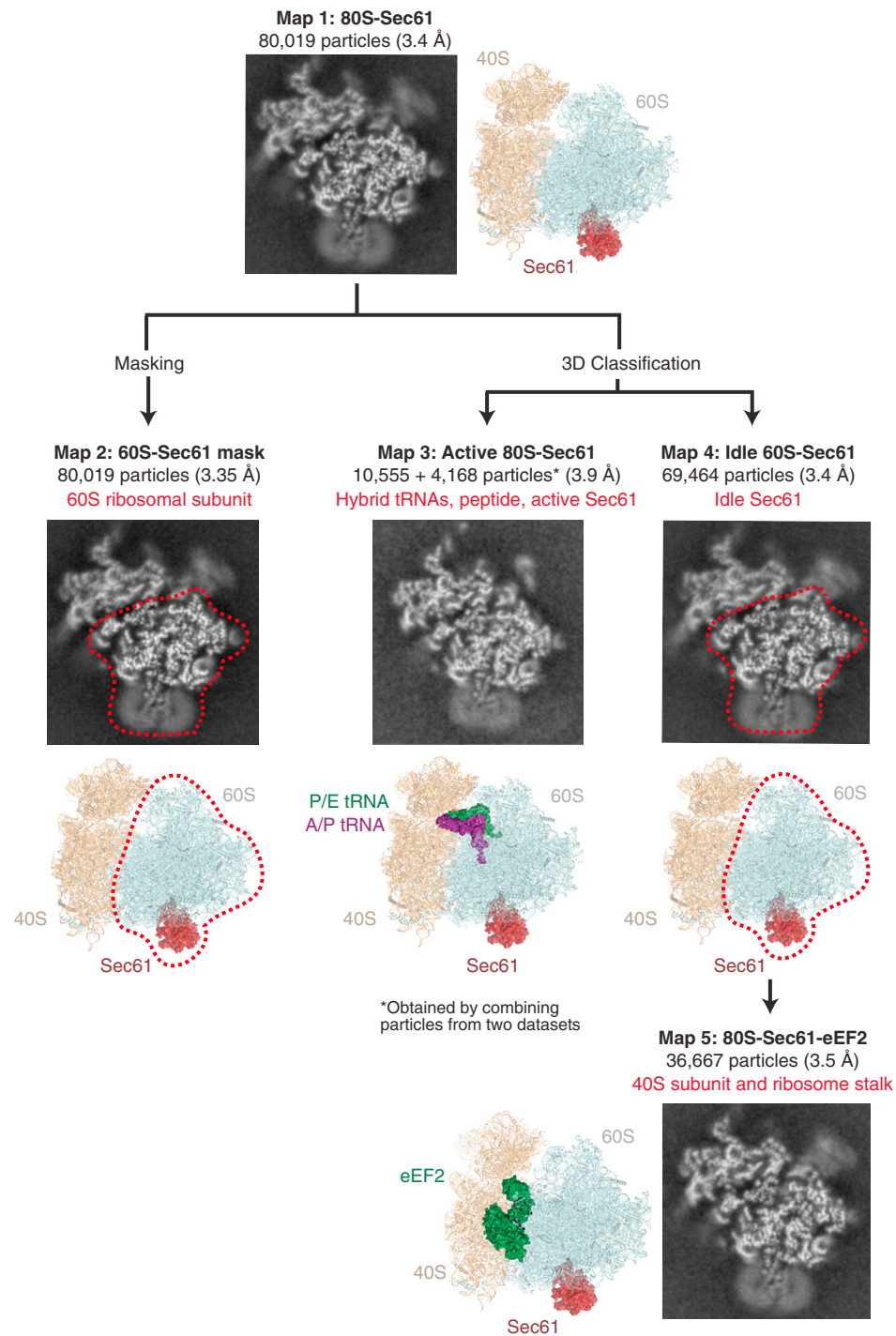
\*Correspondence: [voorhees@mrc-lmb.cam.ac.uk](mailto:voorhees@mrc-lmb.cam.ac.uk) (R.M.V.), [rhegde@mrc-lmb.cam.ac.uk](mailto:rhegde@mrc-lmb.cam.ac.uk) (R.S.H.)

<http://dx.doi.org/10.1016/j.cell.2014.05.024>

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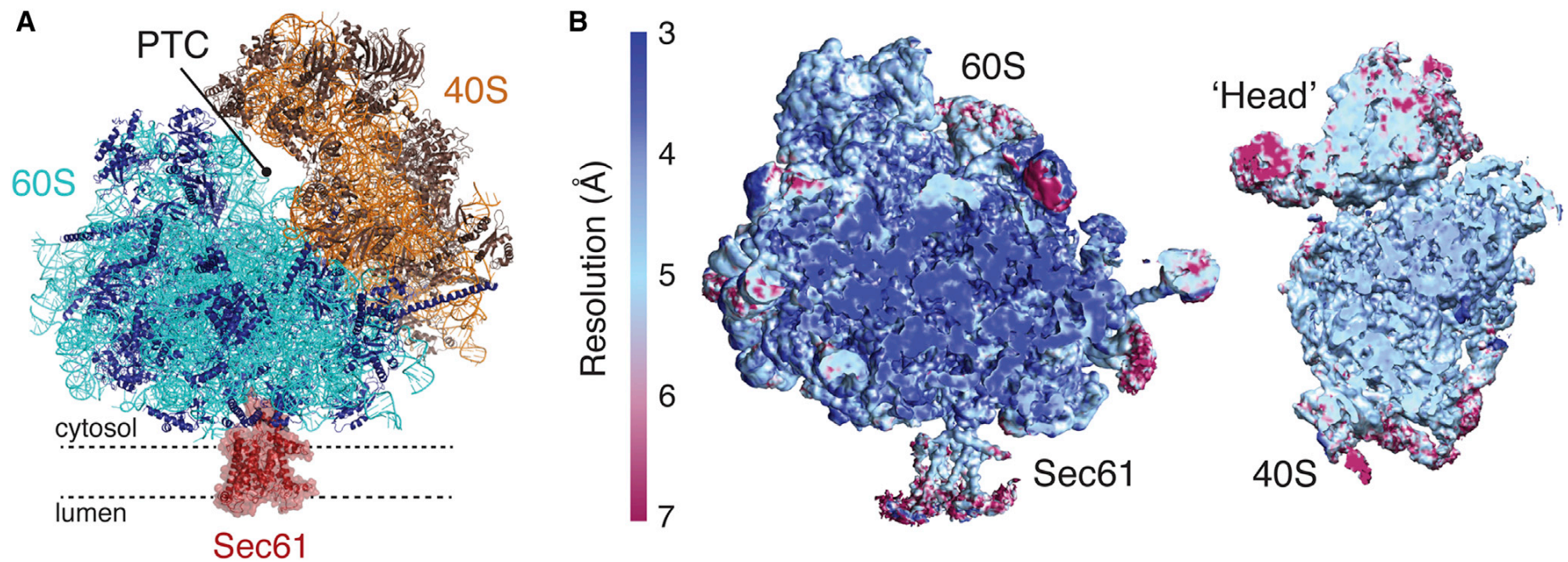






### Figure S2. Refinement and 3D Classification Strategy, Related to Experimental Procedures

Each class in the displayed flowchart shows a cross section image of the respective map, the derived model, any mask that was applied (red dashed line), and the structure (s) derived from that class (red text). The complete data set containing 80,019 particles was refined using RELION ([Scheres, 2012a, 2012b](#)), resulting in an initial reconstruction calculated to 3.4 Å resolution (Map 1). As the 40S subunit was in a variety of distinct conformations, a soft mask for the 60S subunit was used throughout the refinement procedure (Map 2). Although this only resulted in a modest nominal increase in resolution (3.35 Å), the observed density for the 60S subunit was improved compared to the complete refinement, and was used to build the 60S ribosomal proteins and RNA. In parallel, 3D classification of the entire data set identified 13% of particles containing hybrid A/P- and P/E-site tRNAs and a nascent peptide. In order to supplement this relatively small class, an additional data set was collected, resulting in a combined 14,723 particles used to build the translating ribosome-Sec61 complex to 3.9 Å resolution (Map 3). The remaining 87% of particles (69,464) were used to model the idle ribosome-Sec61 complex, lacking a nascent chain and tRNAs, to 3.4 Å resolution (Map 4). Within this idle class, 36,667 particles containing eEF2, which stabilized both the stalk base proteins and the orientation of the 40S subunit relative to the 60S (Map 5). This class of particles was used to build a model for the 40S ribosomal proteins and RNA at 3.5 Å resolution. The remaining idle particles either contained eEF2 bound in different ratcheted conformations (19%) or were devoid of tRNAs or factors (22%). All reported resolutions are determined using the gold-standard Fourier Shell Correlation (FSC = 0.143) criterion ([Scheres and Chen, 2012](#)), and are shown in [Figure S3](#).

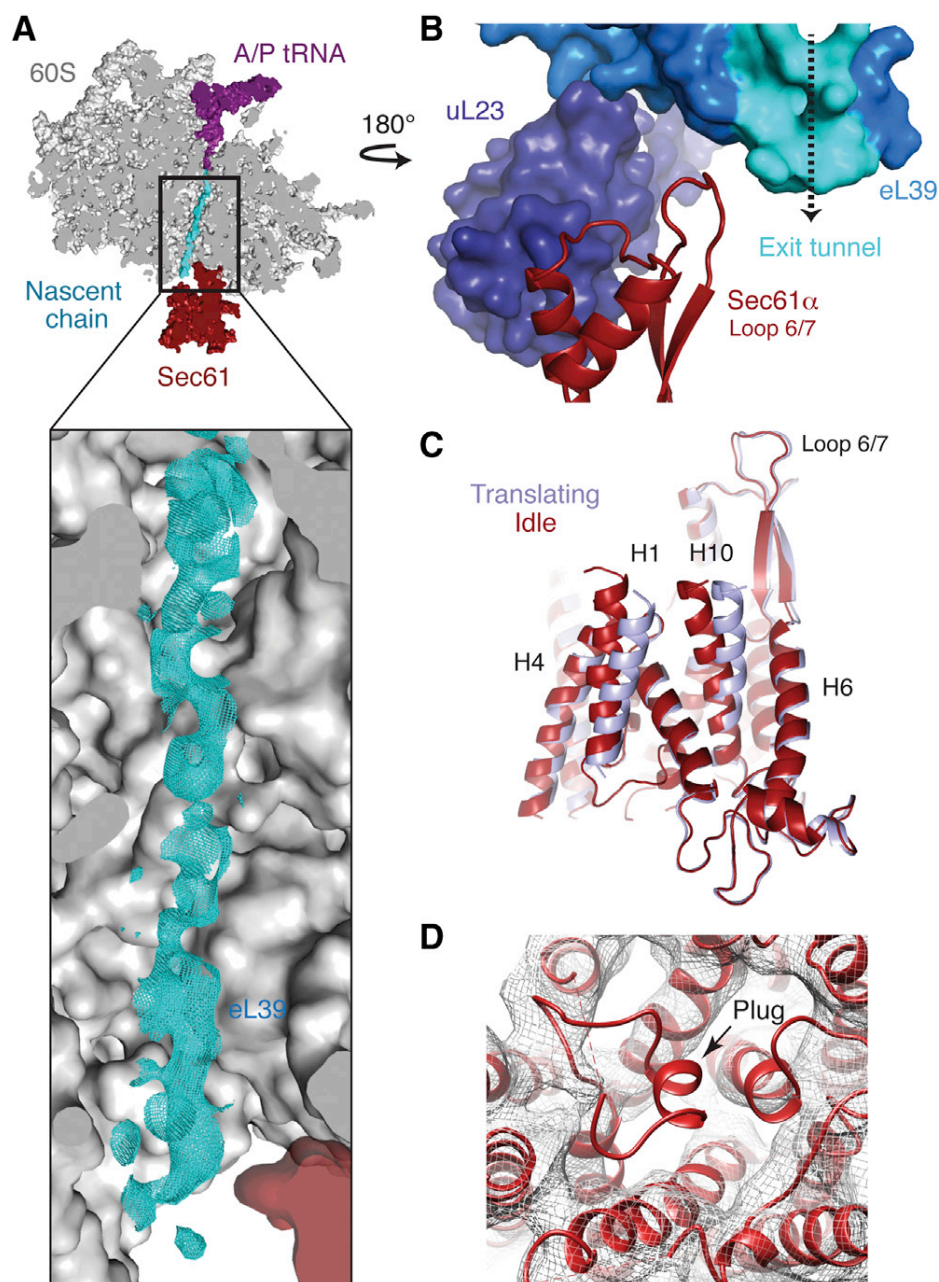


**Figure 1. The Structure of a Mammalian Ribosome-Translocon Complex**

(A) Model of the idle 80S ribosome in complex with Sec61, shown in red. The color scheme shown here is used throughout the manuscript: 40S rRNA is displayed in orange, the 40S ribosomal proteins in brown, the 60S rRNA in cyan, and the 60S ribosomal proteins in dark blue. The region of the peptidyl transferase center (PTC) is indicated.

(B) Cut view of the final unsharpened cryo-EM density map for both the idle 60S-Sec61 complex and the 40S subunit, colored by local resolution in Å (Kucukelbir et al., 2014). Also see Figure S3.





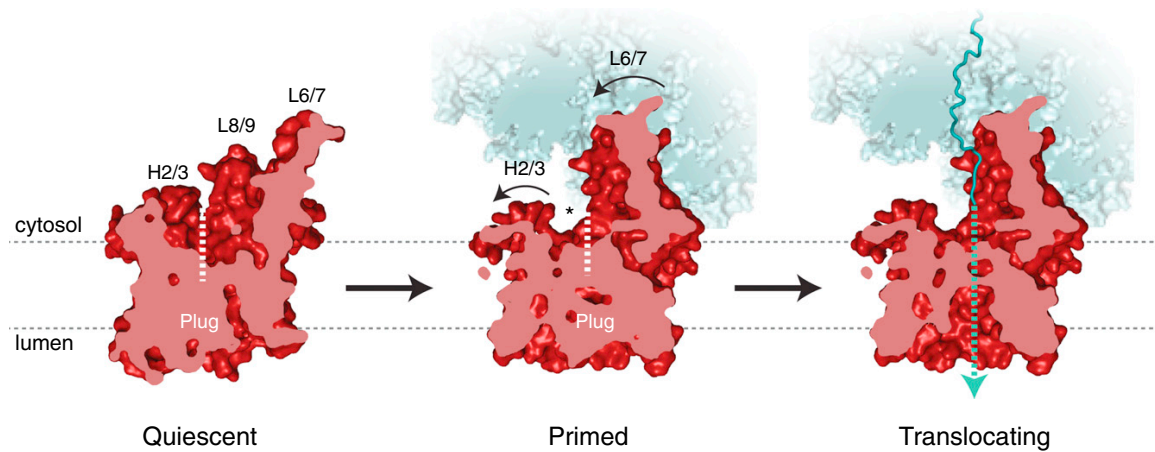
**Figure 6. The Translating Ribosome-Sec61 Complex**

(A) Cryo-EM density within the ribosomal exit tunnel for the nascent peptide (cyan), which spans from the A/P tRNA to Sec61. The location of ribosomal protein eL39, which lines the exit tunnel, is indicated.

(B) Ribosomal protein eL39 (bright blue) forms part of the exit tunnel (highlighted in cyan) and interacts with loop 6/7 of Sec61. Ribosomal protein uL23 (dark blue) contacts both eL39 and loop 8/9 of Sec61.

(C) Comparison of the Sec61 channel structures bound to idle or translating ribosome, showing movements in helices 1 and 10, which may be important for allowing translocation of the nascent polypeptide. Also see Figure S6.

(D) Rigid-body fitting of the idle Sec61 model (red) into the density for the translating Sec61-ribosome complex demonstrates that the plug is not visible in its canonical location. Displayed is an unsharpened map in which the disordered density for the detergent micelle has been removed using Chimera (Goddard et al., 2007).

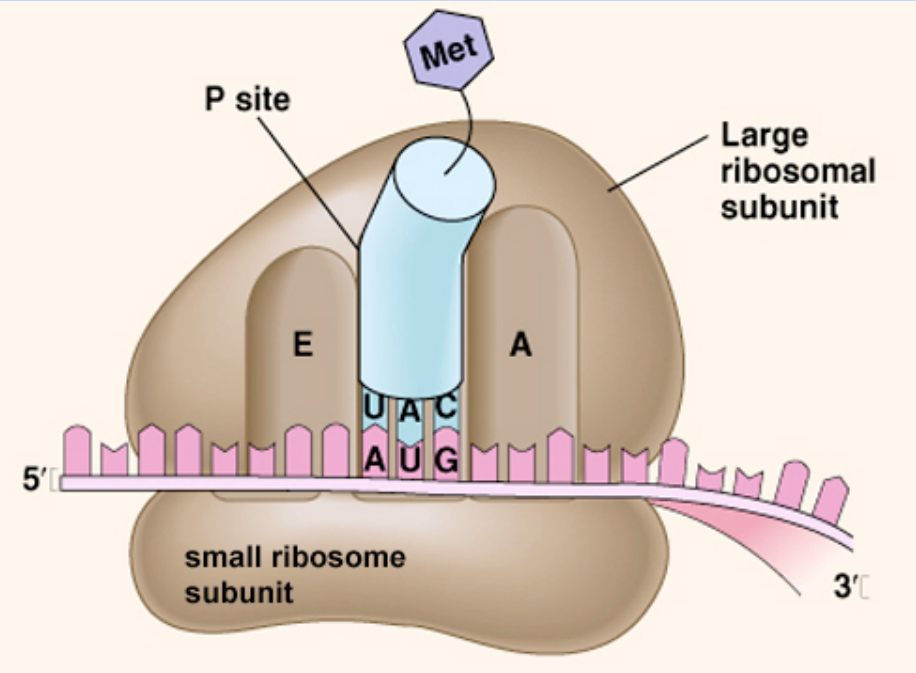
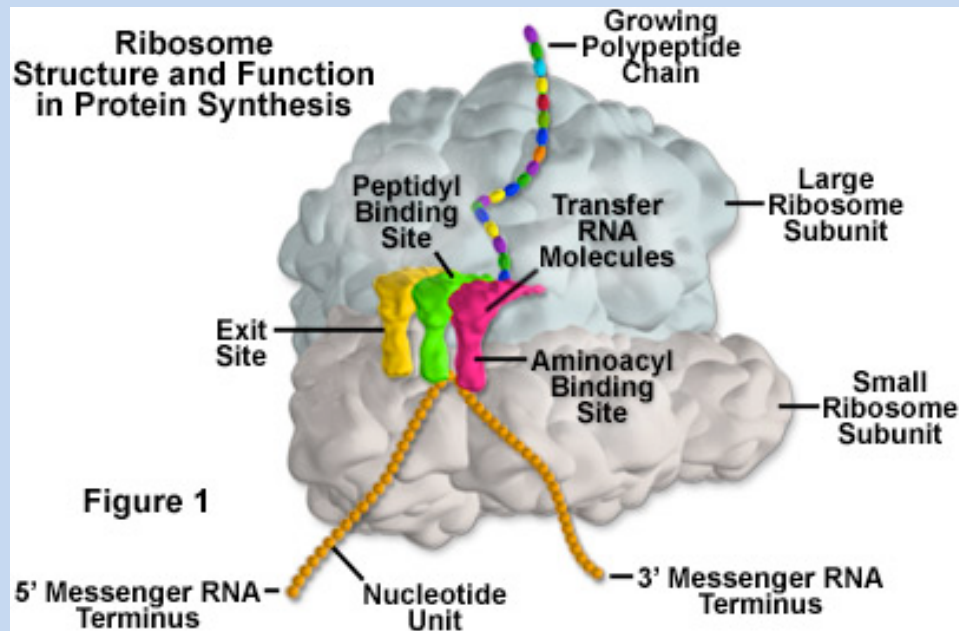


**Figure 7. A Two-Step Model for Activation of Sec61**

Displayed here is a cut-away view of the model for the Sec channel from the central pore toward the lateral gate (dashed line). In the quiescent state (left), approximated by a crystal structure of the archaeal SecY complex ([Van den Berg et al., 2004](#)), the Sec channel is closed to both the lumen and lipid bilayer. The channel becomes primed for protein translocation upon ribosome binding (middle), triggering conformational changes in Sec61 that crack the cytosolic side of the lateral gate (demarcated by an asterisk). The movements of helices 2 and 3 in this region may create an initial binding site for signal peptide recognition.

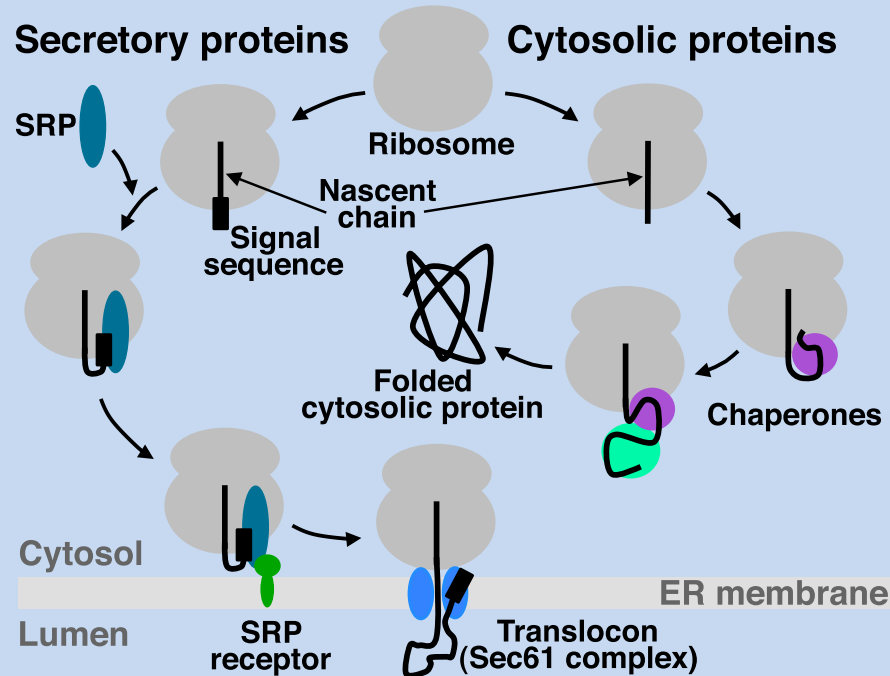
Engagement of the lateral gate by the signal peptide would open the channel toward the membrane and initiate translocation (not depicted; [Park et al., 2014](#)). The translocating state of the active ribosome-Sec61 complex (right) contains a nascent polypeptide (teal) and is characterized by a dynamic plug domain and an open conduit between the cytosol and lumen (teal dotted line).

## Ribosome Structure and Function in Protein Synthesis



## Secretory proteins

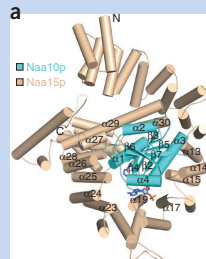
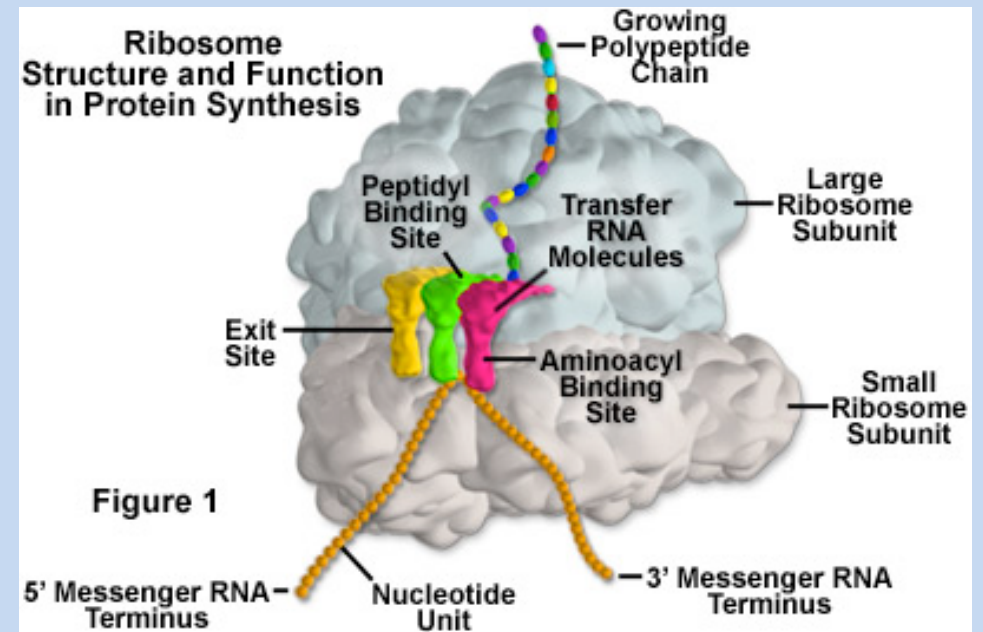
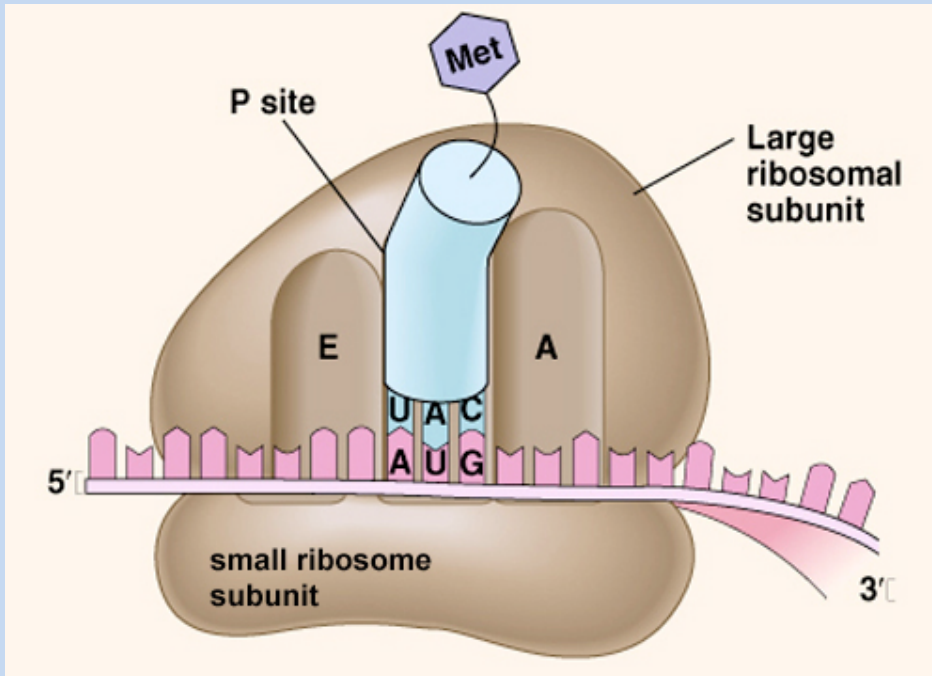
## Cytosolic proteins



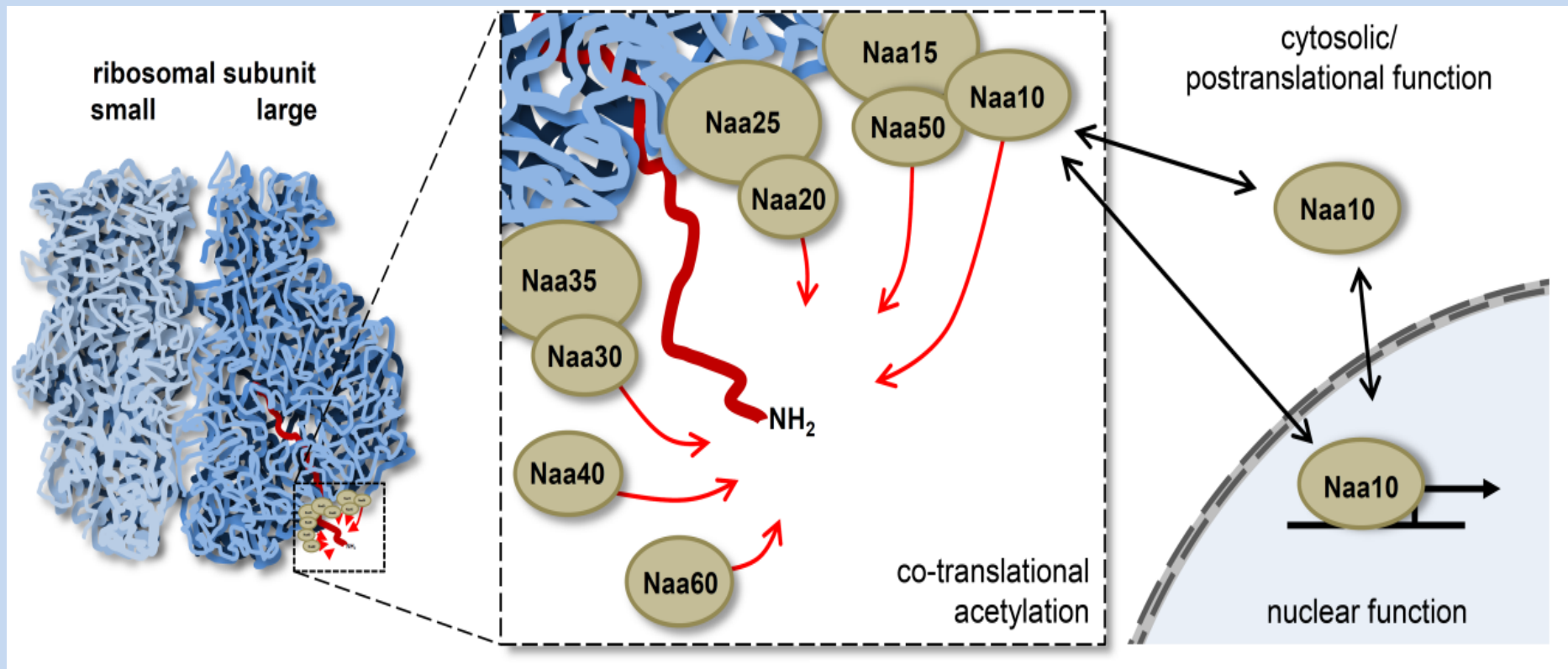
## Inefficient SRP Interaction with a Nascent Chain Triggers a mRNA Quality Control Pathway

Andrey L. Karamyshev,<sup>1,\*</sup> Anna E. Patrick,<sup>1,9</sup> Zemfira N. Karamysheva,<sup>1</sup> Dustin S. Griesemer,<sup>1,10</sup> Henry Hudson,<sup>1</sup> Sandra Tjon-Kon-Sang,<sup>1</sup> IngMarie Nilsson,<sup>3</sup> Hendrik Otto,<sup>5</sup> Qinghua Liu,<sup>2</sup> Sabine Rospert,<sup>5</sup> Gunnar von Heijne,<sup>3,4</sup> Arthur E. Johnson,<sup>6,7,8</sup> and Philip J. Thomas<sup>1,\*</sup>





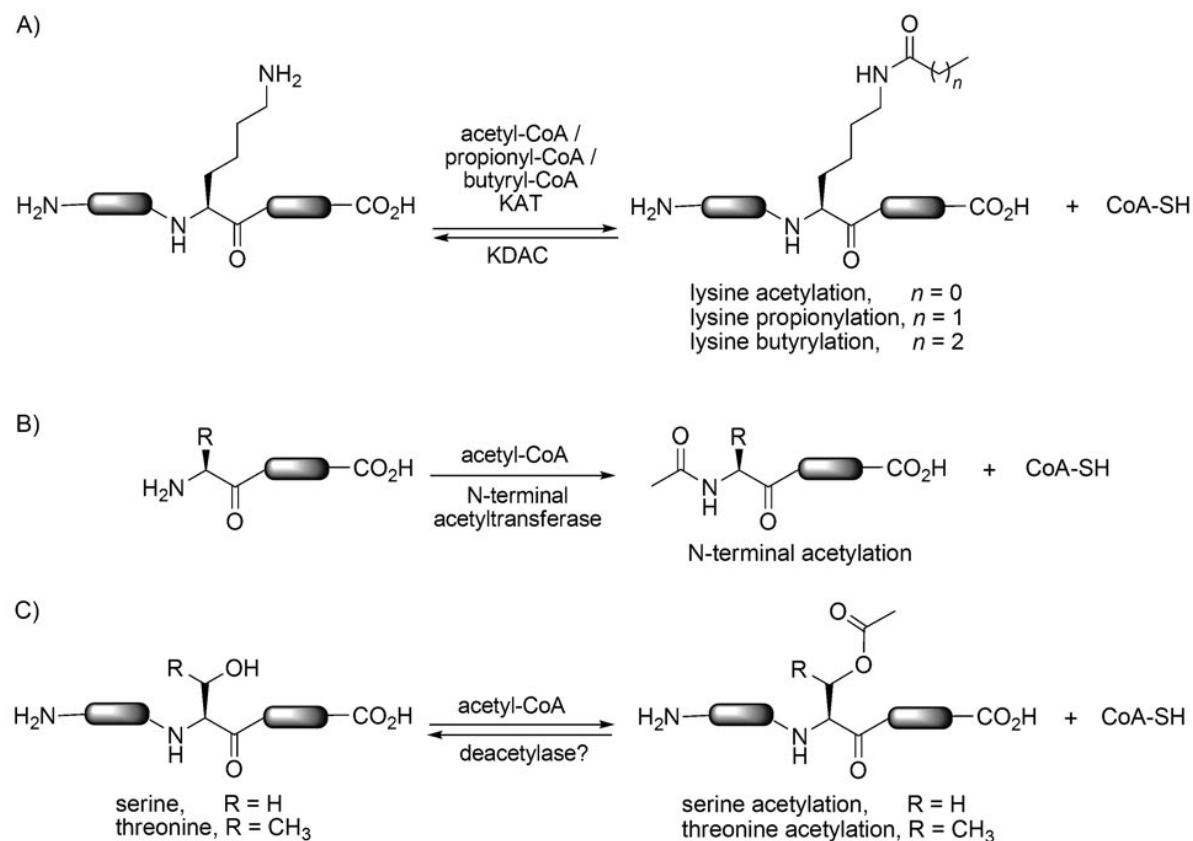
NAA10 + NAA15



- Naa10 co-translationally acetylates the N<sup>α</sup>-terminal amino group of the nascent polypeptide chains of classical substrates as they emerge from the ribosome.

# Chemical Approaches for the Detection and Synthesis of Acetylated Proteins

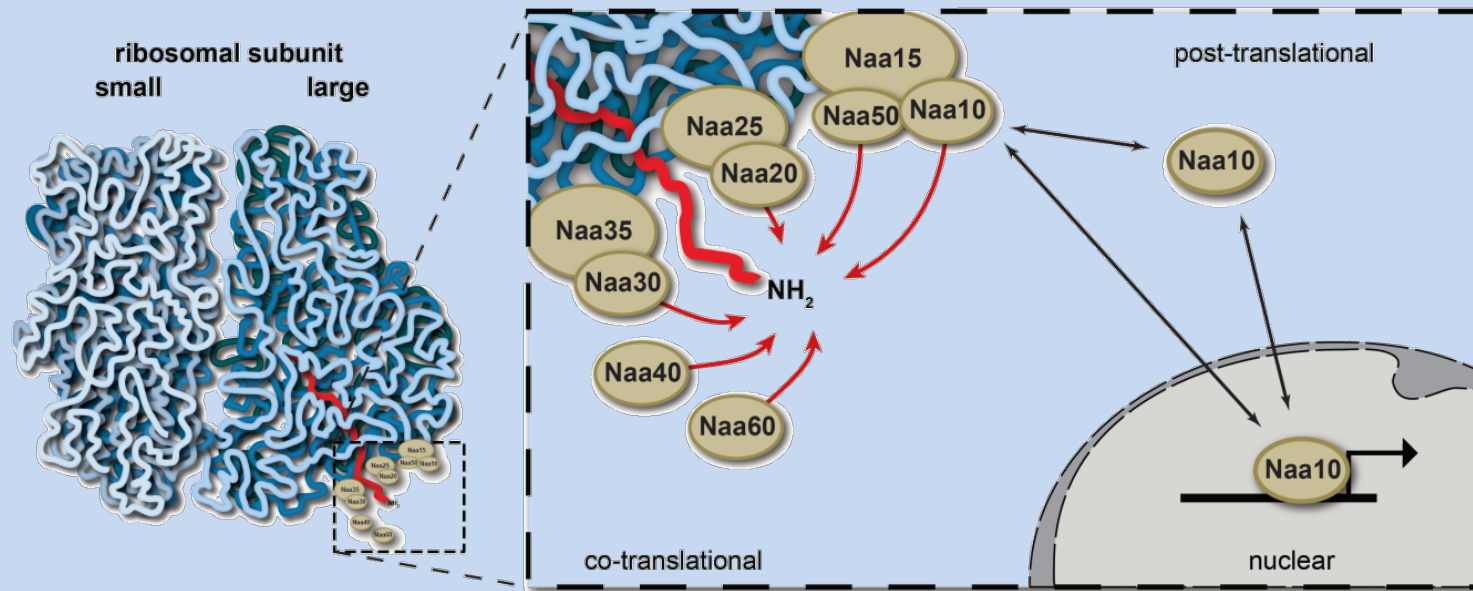
Yu-Ying Yang and Howard C. Hang<sup>\*[a]</sup>



**Scheme 1.** Different classes of protein acetylation. A) Lysine acetylation is a reversible PTM that is tightly regulated by KATs and KDACs. Propionylation and butyrylation have also been described on lysine residues. B) N-terminal acetylation is regulated by N-acetyltransferases. C) Serine and threonine acetylation are executed by secreted bacterial effector proteins during infection of eukaryotic cells.



# NatA (Naa10/Naa15)

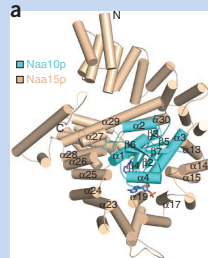


## NatA consists of

- Naa15 (auxiliary subunit; 100 kDa)
- Naa10 (catalytical subunit; 26 kDa)
- Naa50 (catalytical subunit NatE)
- HYPK
- others?

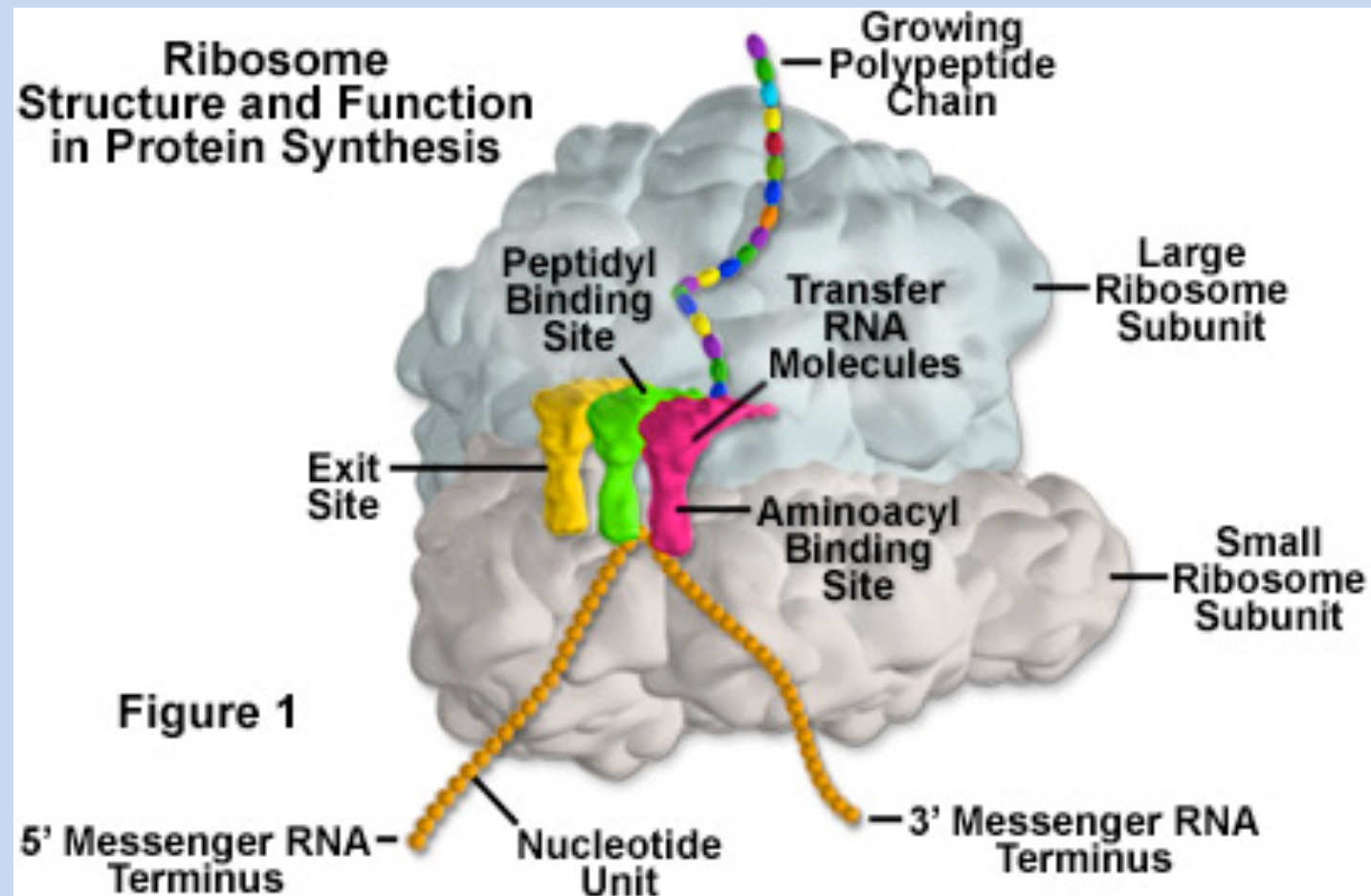
## NatA function

- generation and differentiation of neurons
- $\beta$ -Catenin transcriptional activity
- cyclin D1 regulation
- NF- $\kappa$ B /DNA-damage response pathways
- cellular hypoxia
- apoptosis & cancer

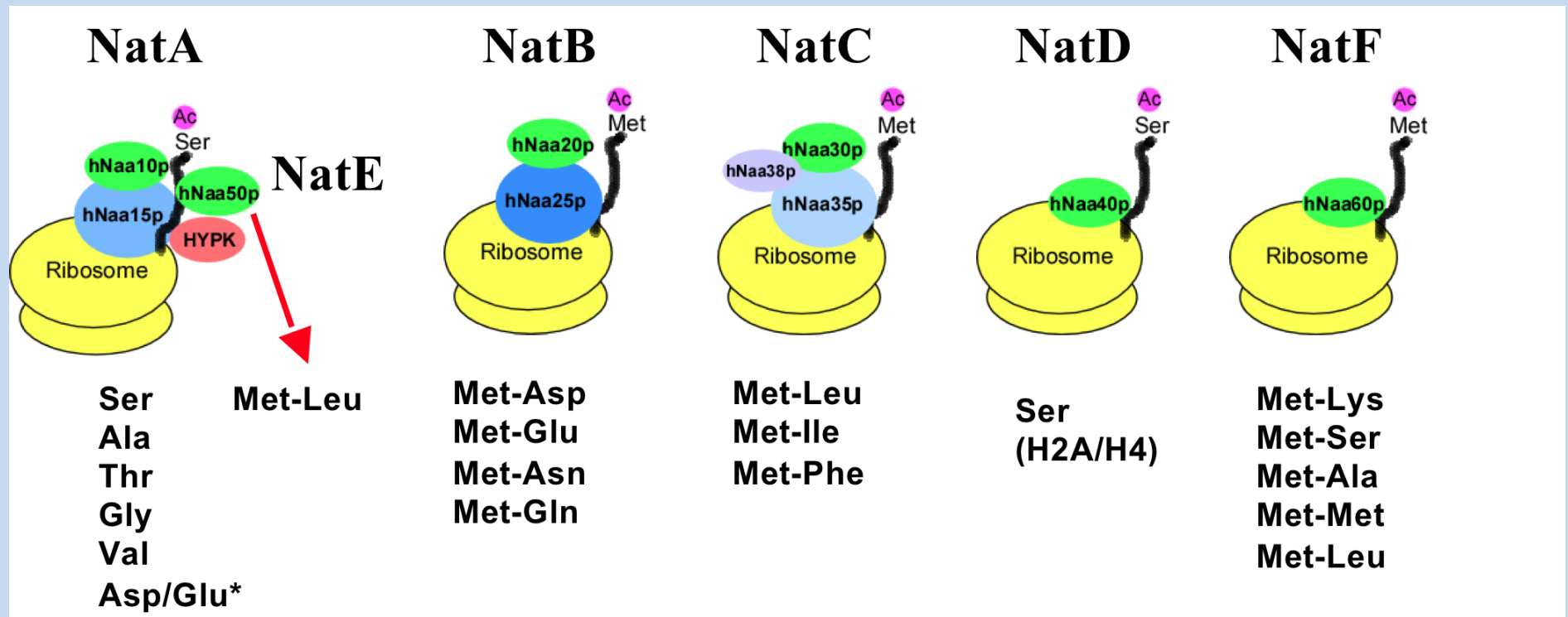


NAA10 + NAA15

And MAPs and SRP and chaperones

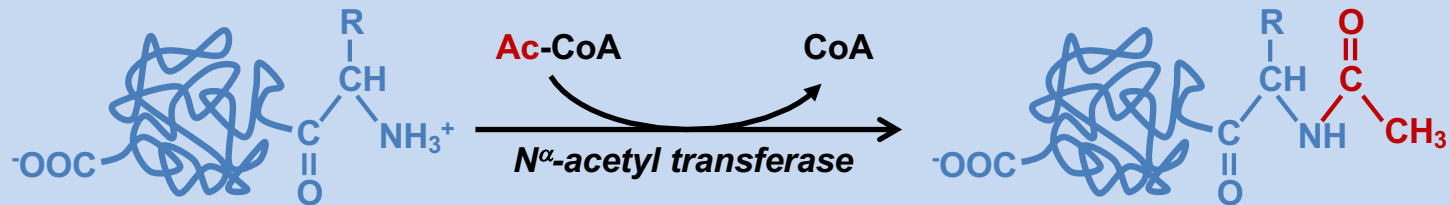


# N-terminal acetylation machinery (NatA) in human cells.



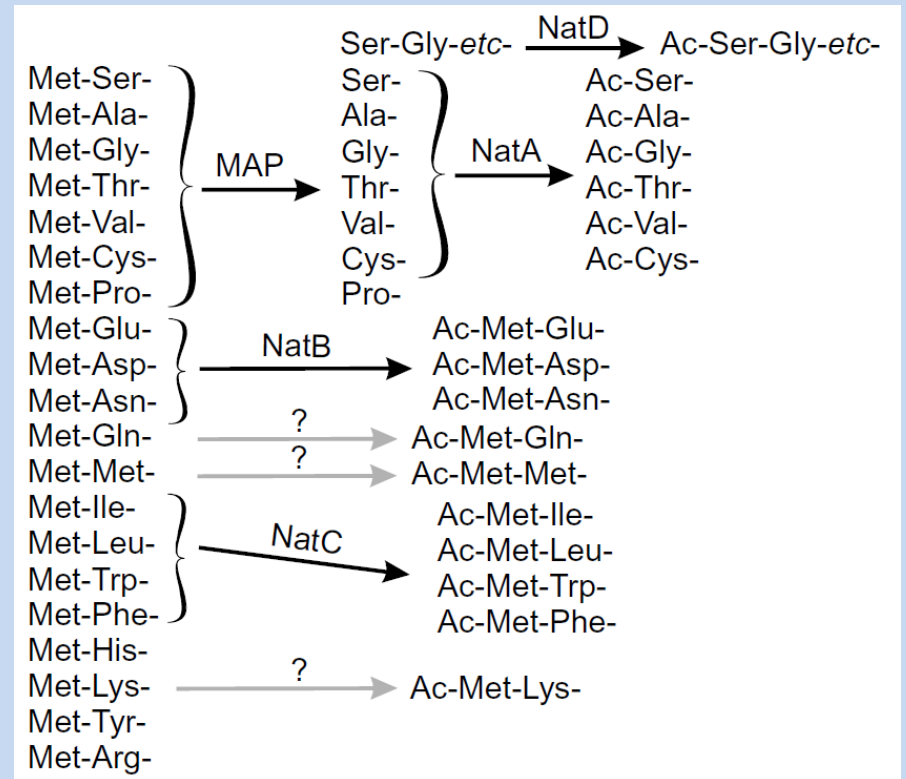
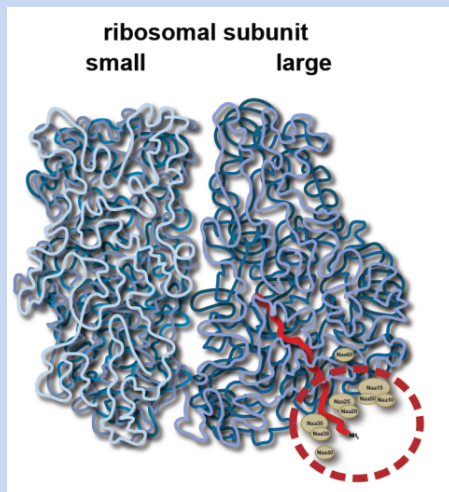


# N<sup>α</sup>-terminal acetyltransferases

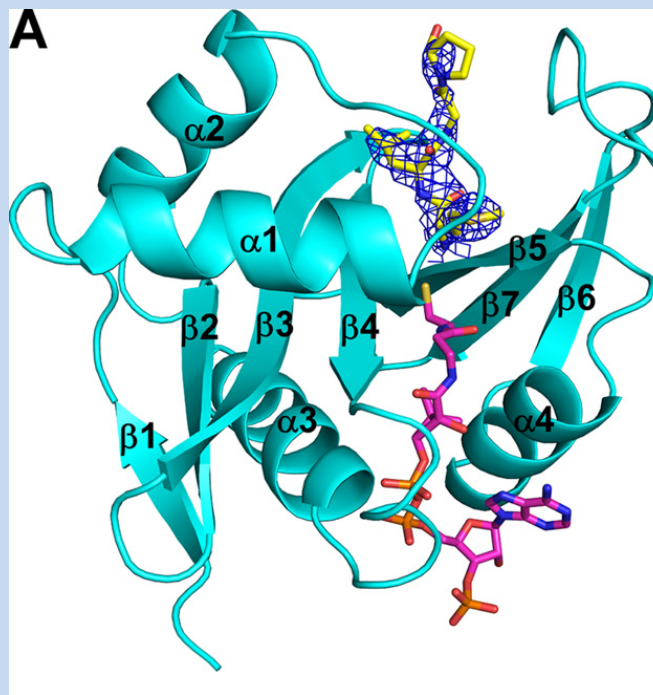


## human NATs

- NatA-NatF
- associated with ribosome
- act co-translationally
- distinct substrate specificity



# Crystal Structure of NAA50



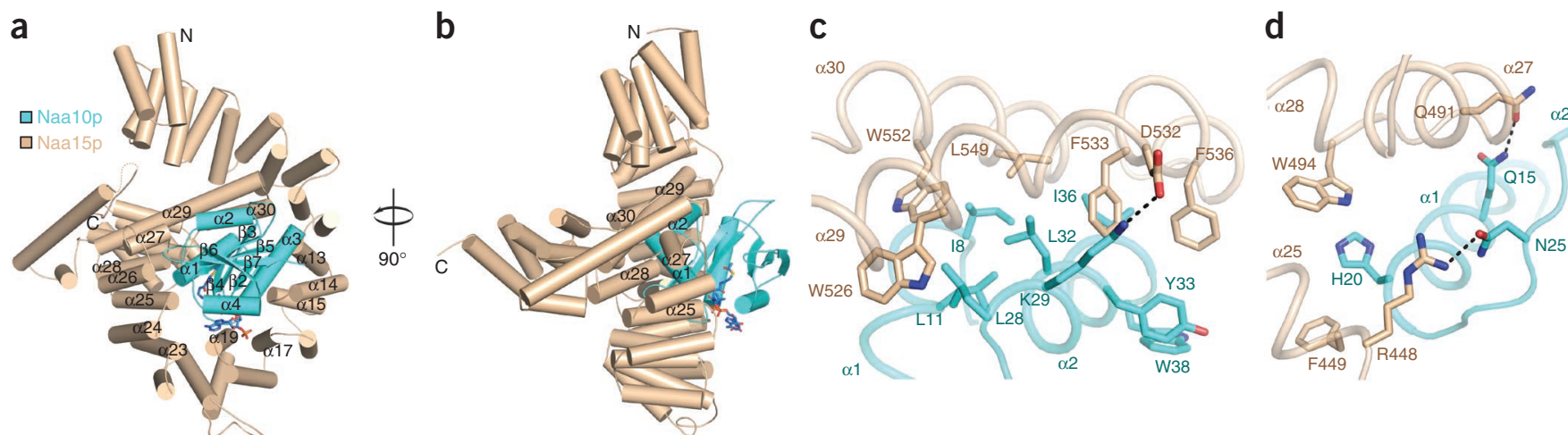
**FIGURE 1. Overall structure of the ternary Naa50p·CoA·peptide complex.** *A*, structure of the ternary complex showing Naa50p in *teal*; CoA as *magenta sticks* with carbon, nitrogen, and sulfur atoms in Corey-Pauling-Koltun coloring; and the substrate peptide as *yellow sticks* with carbon, nitrogen, and sulfur atoms in Corey-Pauling-Koltun coloring. Substrate peptide electron density obtained from a composite omit map (*blue*) is shown contoured to  $1.5\sigma$ . *B*, superposition of the ternary Naa50p complex with the ternary Gcn5

**Glen Liszczak<sup>‡§</sup>, Thomas Arnesen<sup>¶||</sup>, and Ronen Marmorstein<sup>‡§1</sup>**

# Molecular basis for N-terminal acetylation by the heterodimeric NatA complex

Glen Liszczak<sup>1,2</sup>, Jacob M Goldberg<sup>2</sup>, Håvard Foyn<sup>3</sup>, E James Petersson<sup>2</sup>, Thomas Arnesen<sup>3,4</sup> & Ronen Marmorstein<sup>1,2</sup>

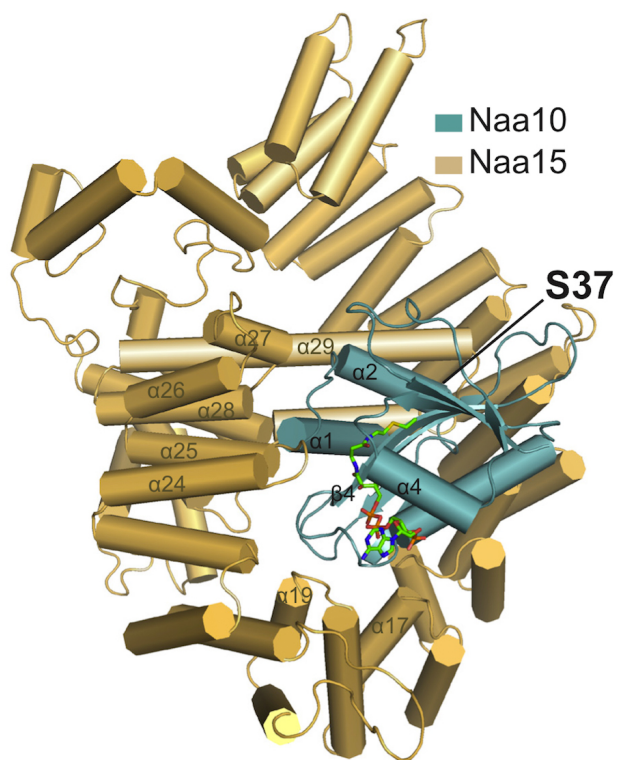
VOLUME 20 NUMBER 9 SEPTEMBER 2013 NATURE STRUCTURAL & MOLECULAR BIOLOGY



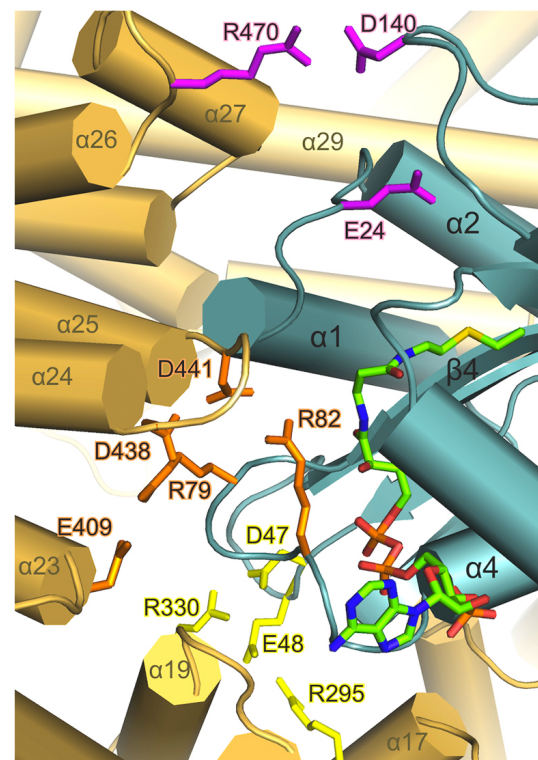
**Figure 1** Overall structure of the NatA complex bound to acetyl CoA. **(a)** Naa10p (teal) and Naa15p (brown) subunits, shown in cartoon, bound to acetyl CoA (CPK coloring and stick format). Only Naa15p helices that contact Naa10p are labeled. The dashed brown line represents a disordered loop region in Naa15p. The dimensions of the complex are 107 Å × 85 Å × 70 Å. **(b)** A 90° rotation of the view in **a**. Helices that are depicted in **c** and **d** are labeled. **(c)** Zoom view highlighting key residues that compose the predominantly hydrophobic interface between Naa10p α1-α2 and Naa15p α29-α30. **(d)** Zoom view of the intersubunit interface at the C-terminal region of Naa10p α1 and the Naa15p α25-α27-α28 helices.



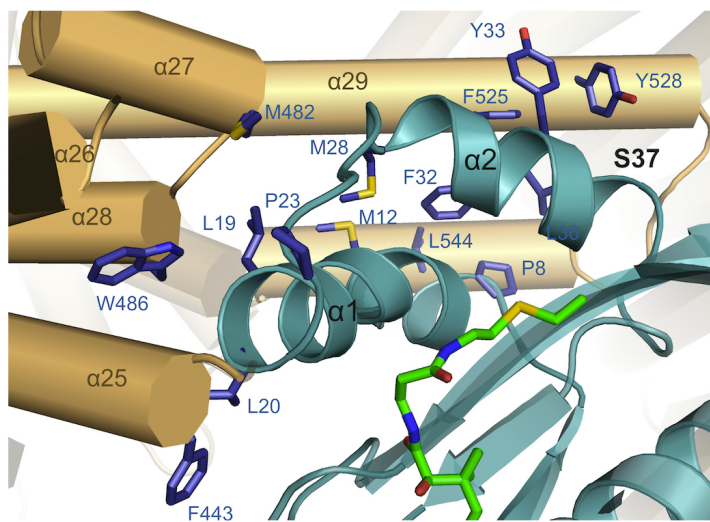
a



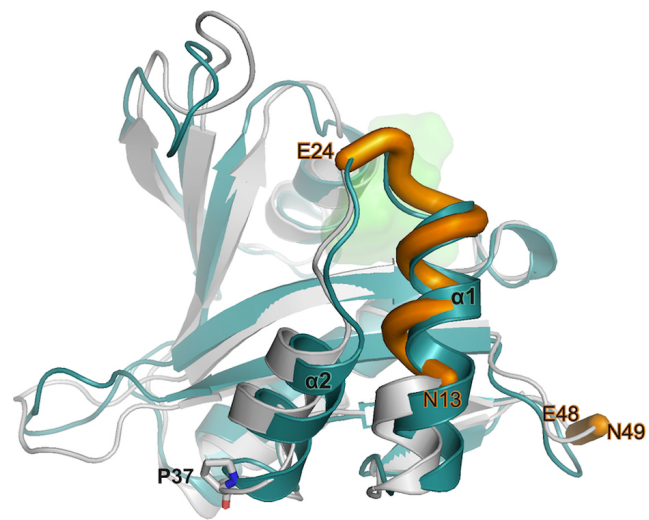
b



c



d



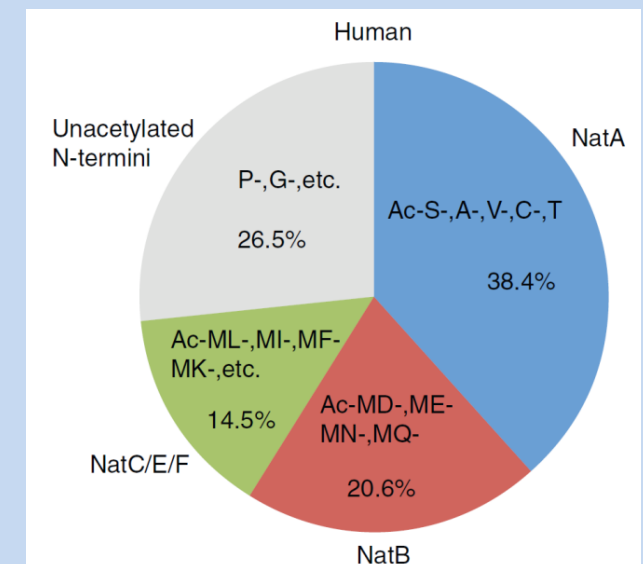
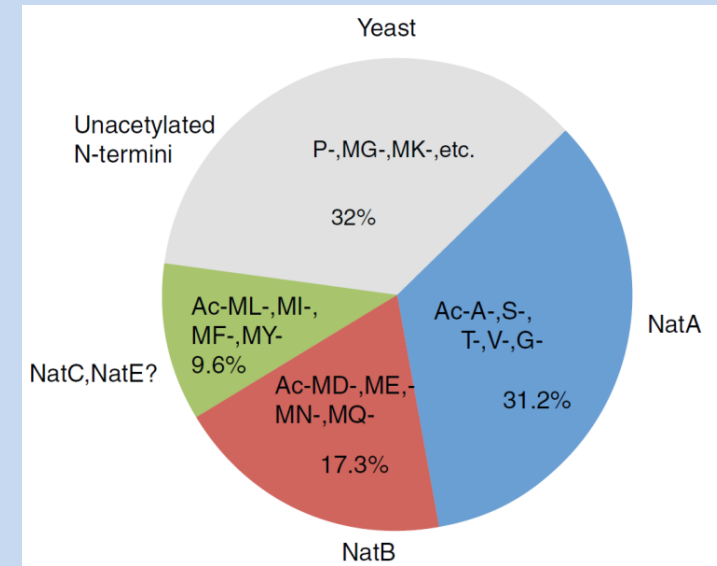
# function of N<sup>α</sup>-terminal acetylation

## general

- most abundant protein modification in eukaryotes
- NatA is the major NAT

## NAT function

- protein function (hemoglobin, actin/tropomyosin...)
- protein stability
- proteasomal degradation via ubiquitin ligase Doa10
- avidity enhancer
- protein targeting to ER



# Open question: Function of N-terminal acetylation?

Protein stability? Protein secretion?

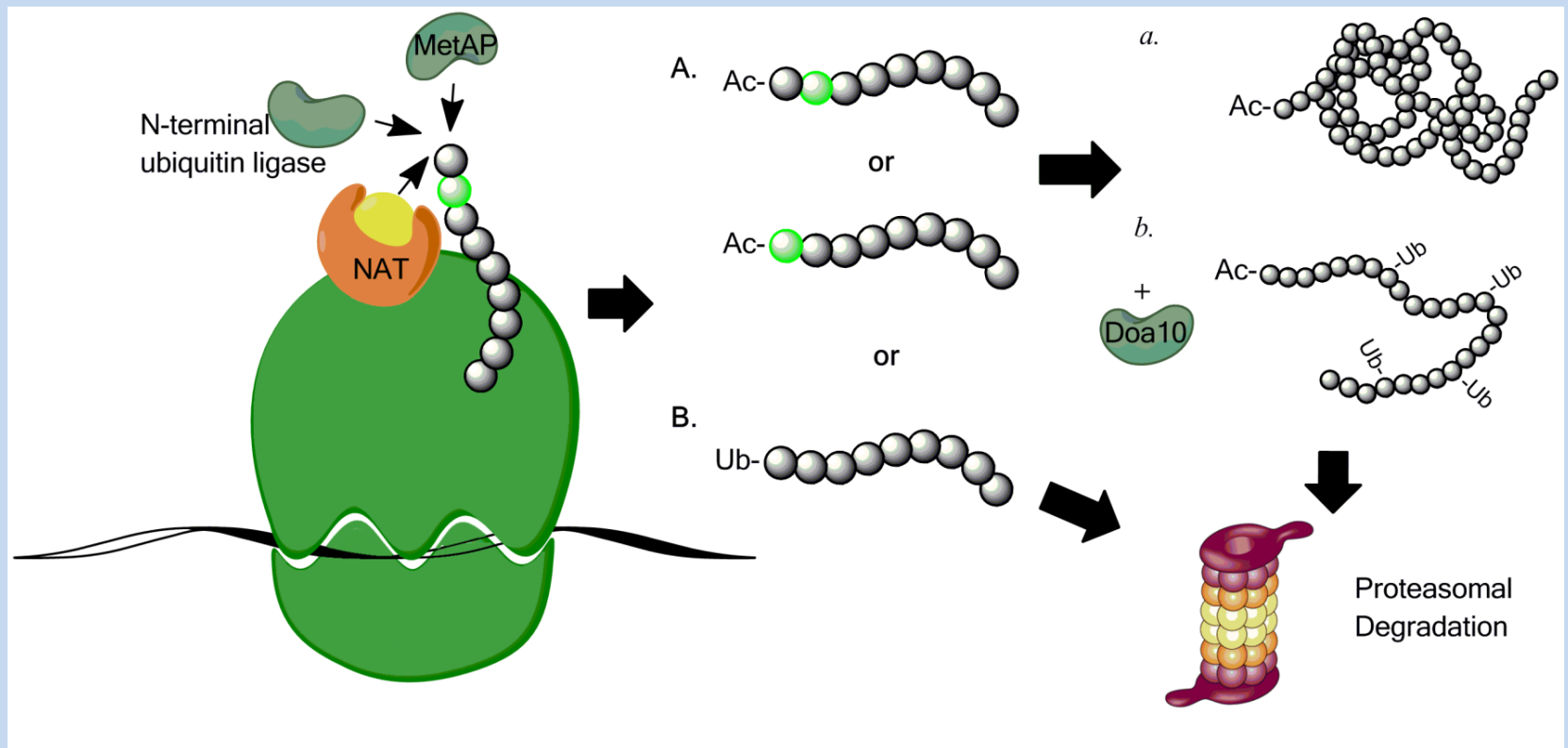


Figure courtesy of Kris Gevaert



# N-Terminal Acetylation of Cellular Proteins Creates Specific Degradation Signals

Cheol-Sang Hwang, Anna Shemorry, Alexander Varshavsky\*

## N-Terminal Acetylation Inhibits Protein Targeting to the Endoplasmic Reticulum

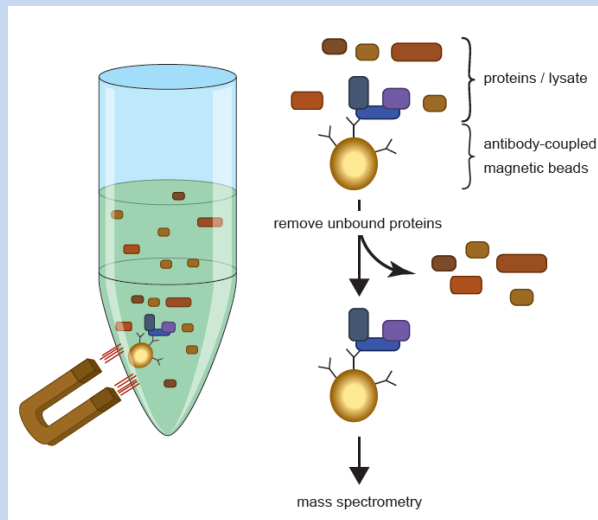
Gabriella M. A. Forte, Martin R. Pool\*, Colin J. Stirling\*

Faculty of Life Sciences, University of Manchester, Manchester, United Kingdom

### Abstract

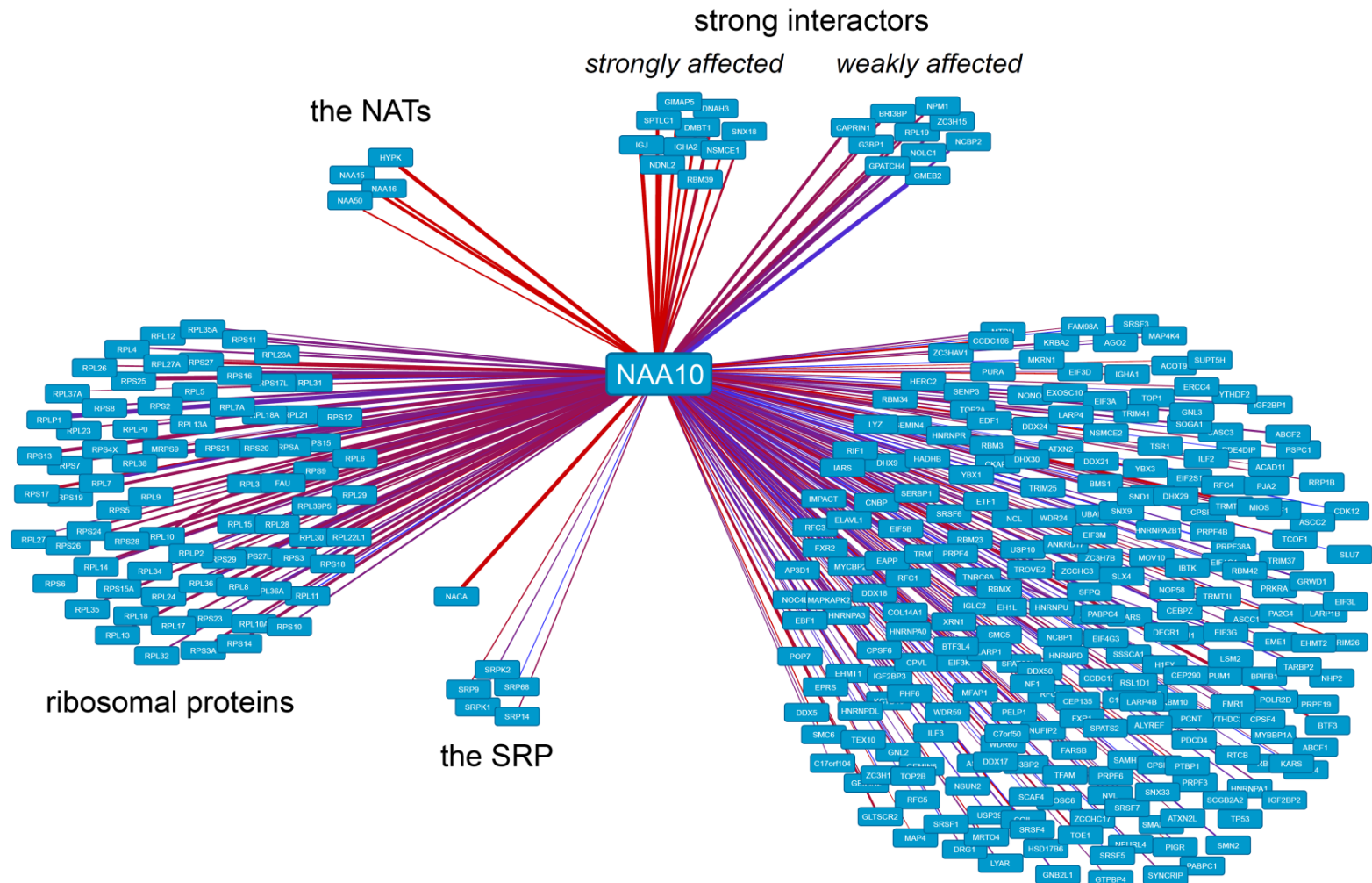
Amino-terminal acetylation is probably the most common protein modification in eukaryotes with as many as 50%–80% of proteins reportedly altered in this way. Here we report a systematic analysis of the predicted N-terminal processing of cytosolic proteins versus those destined to be sorted to the secretory pathway. While cytosolic proteins were profoundly biased in favour of processing, we found an equal and opposite bias against such modification for secretory proteins. Mutations in secretory signal sequences that led to their acetylation resulted in mis-sorting to the cytosol in a manner that was dependent upon the N-terminal processing machinery. Hence N-terminal acetylation represents an early determining step in the cellular sorting of nascent polypeptides that appears to be conserved across a wide range of species.

# materials/methods IP mass spec



5 x 10<sup>6</sup> HEK293 cells were seeded in 10 cm dish and transfected after 24 h with pcDNA3.1 V5/His hNaa10 wt, pcDNA3.1 V5/His hNaa10 S37P or corresponding empty vector. Cells were lysed after 48 h in 500  $\mu$ l lysis buffer (0.2 % Triton X-100, 0.1 % DTT and 1 x Complete protease inhibitor cocktail in PBS) per dish and cellular debris was pelleted at 20,800 g for 10 min. The lysates were pooled and protein concentration was determined using APA protein assay (Cytoskeleton). For IP, 40 mg total protein were incubated with 400  $\mu$ l anti-V5-coupled magnetic beads (Invitrogen) for 2 h at 4°C under constant agitation. Beads were washed 3 times with lysis buffer, 1 x with PBS and once with 50 % PBS. **Proteins were digested with trypsin off the beads and labelled with distinct isobaric iTRAQ reagents.** The samples were combined and subjected to standard 2D MudPIT LCMS and analyzed using a Thermo Velos Orbitrap mass spectrometer. Peptides were fragmented using the high-energy collision (HCD) cell to avoid low-mass ejection of iTRAQ reporter ions. The effects of compression of iTRAQ ratio values were procedurally minimized by using narrow precursor selection tolerances (1.2Da) and optimized settings for HCD collision energy and transmission efficiency. iTRAQ labeled peptides remain isobaric in MS but following HCD the iTRAQ reporter ions appear as distinct ions in the range 113 to 121 m/z in each MS/MS spectrum. The relative abundance of the peptides (and thus corresponding proteins) were deduced directly from the relative intensities of the corresponding reporter ions. Peak lists were extracted from instrument raw data files using the MASCOT Distiller software (MatrixScience) and the MASCOT searches were performed on a 64-processor cluster (Perkins et al. 1999). False discovery rates (FDR) were estimated by searching equivalent reversed or randomized sequence databases, typically using a cutoff value of 1% FDR. Protein-abundance ratios were calculated using intensity weighted averages from matching peptides after outlier removal. **The relative enrichment in the samples were calculated as a ratio of the intensities between the samples and the empty-vector control. The whole experiment was done twice.**

slides IP & mass spec

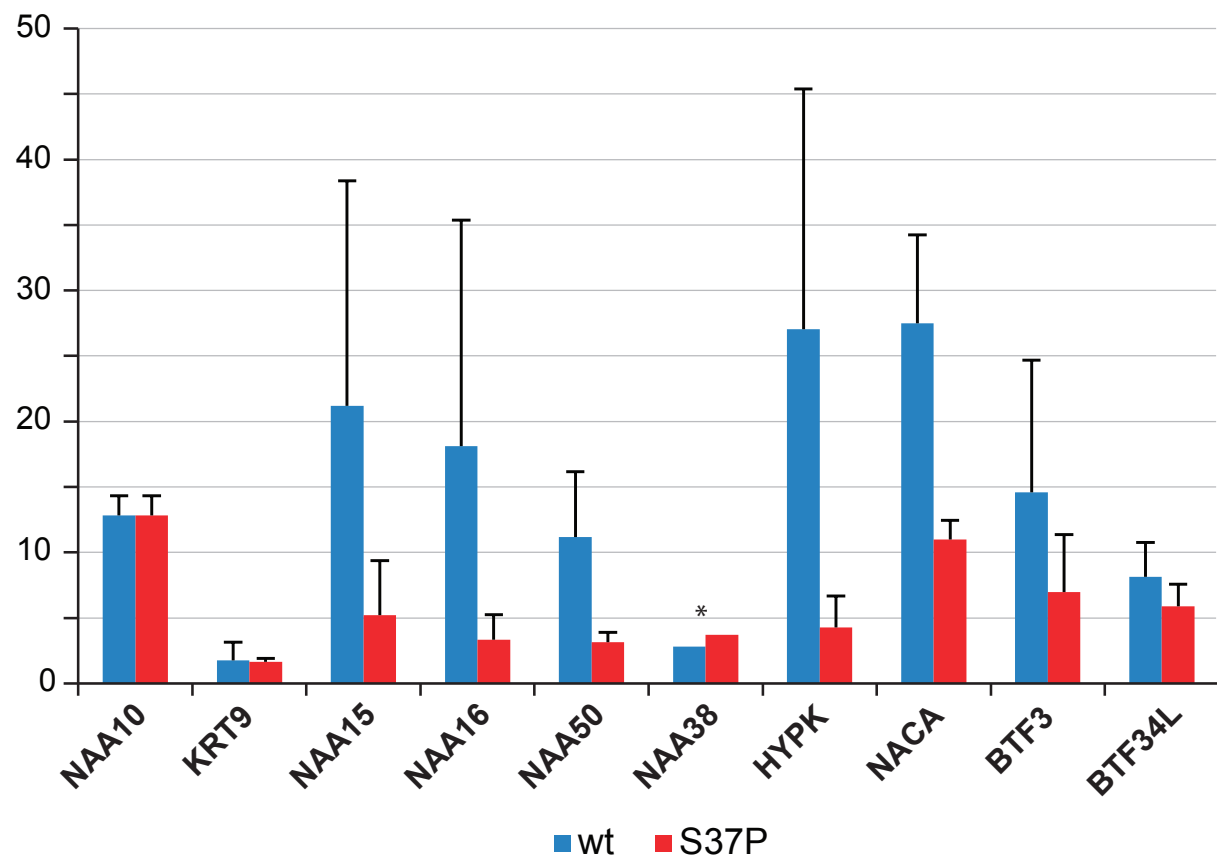




### Top 20 hits

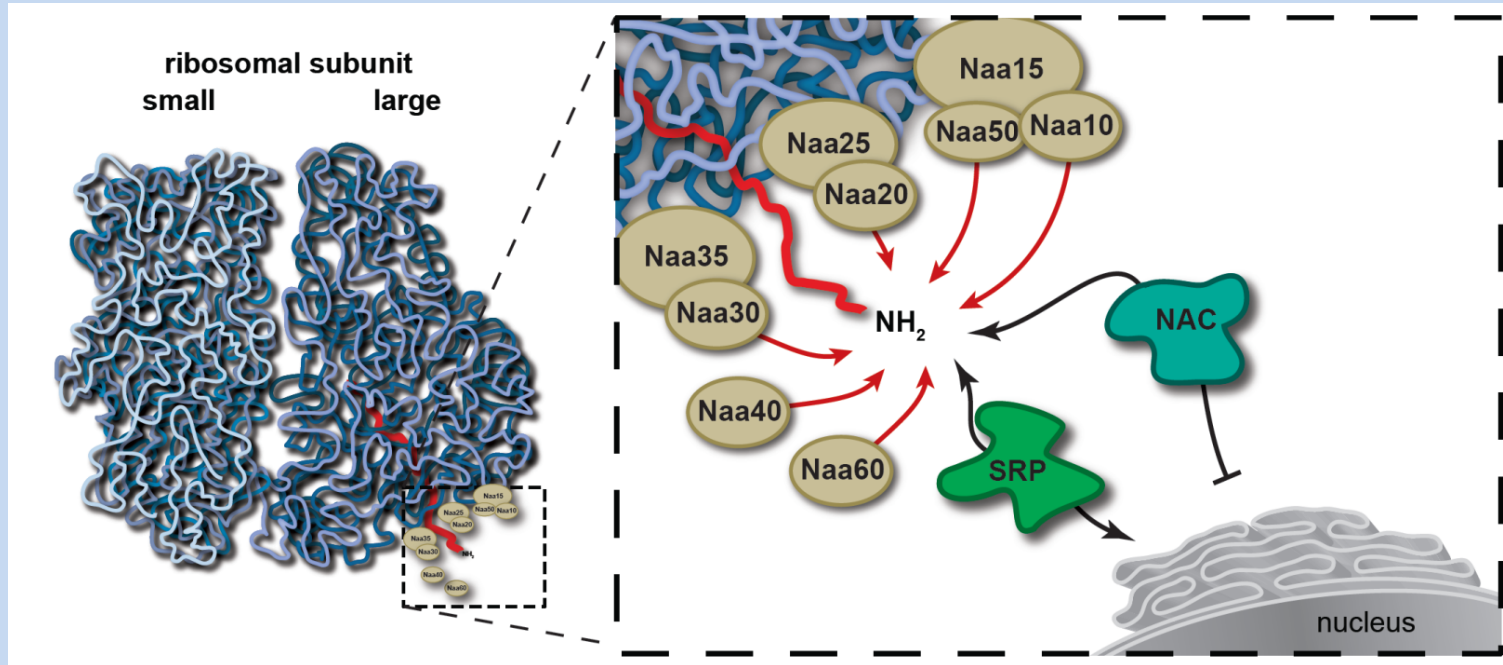
Nr.	Name	Description	Enrichment	
			wt	S37P
1	RPLP1*	60S acidic ribosomal protein P1	30.3	23.7
2	RPS25*	40S ribosomal protein S25	29.4	18.1
3	NACA	Nascent polypeptide-associated complex subunit alpha, muscle-specific form (Fragment)	27.5	11.0
4	HYPK	Huntingtin-interacting protein K	27.0	4.3
5	RPL38*	60S ribosomal protein L38	26.8	18.3
6	RPL8*	60S ribosomal protein L8	24.7	14.4
7	RPS13*	40S ribosomal protein S13	24.1	13.5
8	RPS28*	40S ribosomal protein S28	23.8	14.6
9	FAU*	40S ribosomal protein S30	23.7	15.9
10	RPL27A*	60S ribosomal protein L27a	21.9	11.9
11	RPL6*	60S ribosomal protein L6	21.8	13.7
12	CAPRIN1	Caprin-1	21.7	12.4
13	GPATCH4	G patch domain-containing protein 4	21.4	15.3
14	NAA15	N-alpha-acetyltransferase 15, NatA auxiliary subunit	21.2	5.2
15	RPS18*	40S ribosomal protein S18	20.9	12.0
16	RPS14*	40S ribosomal protein S14	20.7	11.6
17	RPS19*	40S ribosomal protein S19	20.3	12.8
18	RPL13*	60S ribosomal protein L13	19.9	11.5
19	NDNL2	Melanoma-associated antigen G1	19.6	4.7
20	RPS16*	40S ribosomal protein S16	19.5	13.6

\*ribosomal protein



\* Naa38 was only found in one set

# NAC





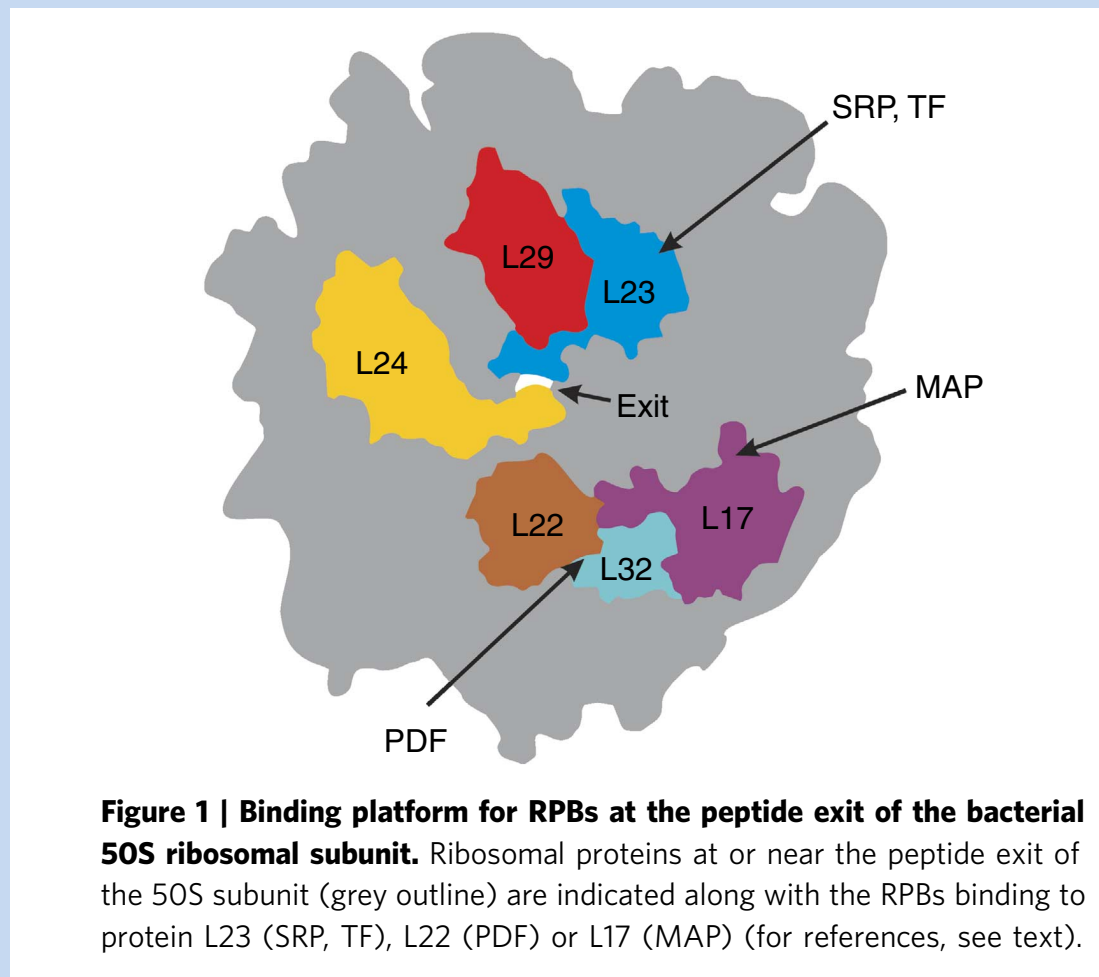
## ARTICLE

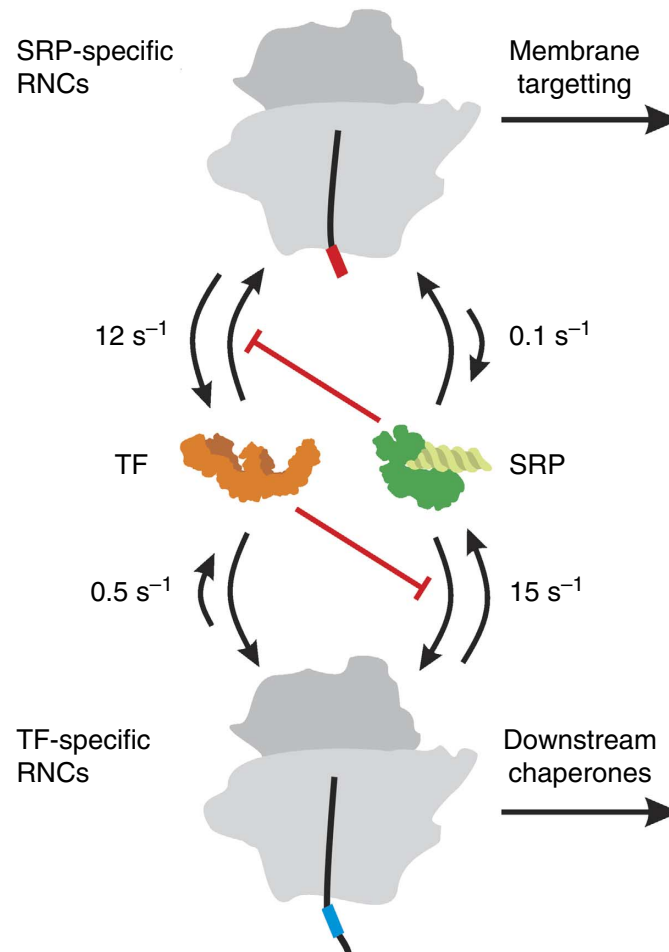
Received 24 Feb 2014 | Accepted 21 May 2014 | Published 18 Jun 2014

DOI: 10.1038/ncomms5180

# Interplay between trigger factor and other protein biogenesis factors on the ribosome

Thomas Bornemann<sup>1</sup>, Wolf Holtkamp<sup>1</sup> & Wolfgang Wintermeyer<sup>1</sup>





**Figure 6 | Interplay of TF and SRP on translating ribosomes.** Concurrent binding of TF or SRP to RNCs presenting the respective specific nascent chain (SRP-specific SAS, red; TF-specific sequence, blue) leads to weakening of the binding of the respective other ligand (values of  $K_d$  increased eightfold or >15-fold, respectively (Fig. 3)), as indicated by red lines. Because SRP dissociates from RNCs in three steps, average values of  $k_{off}$  calculated from the three rate constants  $k_{-1}$ ,  $k_{-2}$  and  $k_{-3}$  are given<sup>13</sup>. Values of  $k_{off}$  for TF dissociation from RNCs are taken from Table 1. Second-order rate constants for TF or SRP binding to RNCs ranged from about 100 to 200  $\mu\text{M}^{-1}\text{s}^{-1}$  (Table 1 and ref. 13), indicating near diffusion-controlled binding.